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ACTINOMYCOSIS AND ACTINOBACILLOSIS.

By SIR JOHN MCFADYEAN.

THE main purpose of this article is to call attention to some prevalent misconceptions with regard to the connotation of these two terms.

Although Veterinary Surgeons in this and other European countries had long been familiar with the gross lesions in the tongue and jaws of cattle which are now covered by the term actinomycosis, it was Bollinger who first gave what may be called a scientific account of the disease, including a description of the organism which he found in the lesions. Harz, the Botanist in the Royal Veterinary College in Munich, to whom specimens were submitted by Bollinger, recognised the vegetable nature of the parasite, and named it the *Actinomyces bovis*, or, literally, the ray fungus, on account of the radiate arrangement of the separate elements in the conglomerate growths found in the diseased tissues.

For reasons which will be more apparent later, special importance attaches to the report of the lecture and demonstration at which Bollinger[1] made the first public announcement of his discovery. In substance it was as follows :—

" On the upper and lower jaws of the ox there are not rarely found tumour-like growths which have their origin from the alveoli of the molar teeth or the spongy tissue of the bones, and lead to enlargement and absorption, and ultimately, after they have caused loosening of the molar teeth and destroyed the normal tissues in the way of their growth, break through the skin or into the mouth or pharynx. The distended maxillary bones show a macerated appearance, like pumice-stone, determined by central osteoporosis and superficial hyperostosis. The most lumpy and conglomerate

growths after a long time frequently undergo puriform or putrid degeneration and become the site of ulcers, abscesses and fistulous passages, and commonly reach the size of a child's head or more. Hitherto these growths have received different names, such as osteosarcoma, and spina ventosa, or they have been regarded as examples of tuberculosis of bone, or as cases of simple chronic ostitis. Still other terms are used by breeders, who dread the disease on account of its frequency and its incurability.

" The disease develops gradually, and, as a rule, affected animals are slaughtered before they become very emaciated in consequence of interference with mastication.

" When one examines such growths in the fresh condition [three specimens were exhibited] they are found to consist of conglomerate nodules, often the size of a walnut or a hen's egg, and united by dense connective tissue. The nodules have a soft consistence, a pale yellow colour, and a juicy, shiny appearance. On the cut surface one sees turbid, mostly yellowish-white, abscess-like, scattered centres, or firmer nodules with a spongy structure. In the fibrous stroma there are numerous gaps and cavities, about the size of a hemp seed, and occupied by a turbid, yellow, thick, and frequently caseous pulp.

" When one scrapes the cut surface with a knife one obtains both in the young soft, and the older and firmer nodules, a purulent or caseous material.

" Microscopic examination shows that the tumours are mostly composed of young or old granulation tissue ; they show a sarcomatous structure, whereas the pulp obtained by scraping is essentially composed of pus corpuscles, granulation and other cells, and a fatty granular detritus. This detritus constantly contains everywhere numerous opaque, faintly yellow bodies, varying in size, often mulberry-like, and occasionally incrusted with lime salts. On accurate examination these bodies are recognised to be true fungi (Pilze). That they are not accidental, but are of pathological importance, is evident from the fact that they are constantly present in parts of the so-called cancer of the jaw (Kiefersarkome), and I could with certainty recognise them also in old specimens preserved in spirit [six cases].

" This remarkable form of mycosis, however, is not confined to the jaw bones of the ox, where the invasion starts from the dental alveoli, for it is also found in the tongue of the ox, in which position this peculiar disease has long been known. In the parenchyma of the tongue one finds more or less numerous nodular deposits, which frequently project above the surface of the mucous membrane, and have a size equal to that of a millet seed or a hemp seed, often even up to that of a cherry, a walnut, or even larger. In the fresh condition these nodules are usually white, or greyish-white, translucent, juicy and shiny. Very often they appear turbid or puriform, and show central softening and are surrounded by a connective tissue capsule ; in correspondence with their sarcomatous or tubercular appearance the nodules, on microscopic examination, show a cellular granulation growth with central puriform softening or caseous degeneration. When the nodules occur near the surface of the tongue destruction of the mucosa is apt to occur, leading to the formation of erosion, ulcers and cicatrices ; in the parenchyma, on the other hand, a secondary interstitial glossitis develops, and this frequently, in spite of partial atrophy of the muscular bundles, automatically leads to great enlargement of the tongue, which, at the same time acquires a wooden hardness. On account of the latter character, in South Germany the disease when it affects the tongue is termed Holzzunge. The disease is found in

both the jaws and the tongue in cattle of any age, and, while it frequently develops in a few weeks, as a rule growth is slow and the disease is always incurable. It may last for months or years until the animals are slaughtered, when, on account of the restricted movement and the enlargement of the tongue, there is serious interference with feeding. *The nodules in the tongue constantly contain the same fungus as is found in the tumours of the jaws.** That this mycosis of the tongue is not a very rare disease is shown by the fact that, within a year, no fewer than six such ox tongues, all affected in the same way, and containing the same fungus, have been sent to me from different parts of Bavaria. Since I have been able to determine the presence of the fungus in five other museum preparations, there can be no doubt of the ætiological rôle of the organism.

" Of great interest is the further fact that this fungus is found, not only in the centre of the tongue nodules, but also in the lymphatic glands of the tongue and throat, and in the upper cervical lymphatic glands. These are found to be greatly enlarged and converted into spongy grey or dirty yellow nodules, in the cavities of which the fungus is abundantly present. I have no doubt that in the mycosis of the jaws the corresponding lymphatic glands are similarly affected."

It should be noted that the major part of this description was devoted to the tumour-like lesions affecting the jaws and the soft tissues of the head, that no mention is made of any attempt to induce the actinomyces to grow outside the body, and that the finer details of the structure of the fungus granules or colonies are not described.

In 1878, J. Israel[2] described and figured a new vegetable parasite found by him in pus from a case of chronic pyæmia in a woman, but did not immediately identify it with the one described by Bollinger.

In 1882 Johne[3] contributed a very important article on " the new disease."

Commencing with a quotation from the above lecture by Bollinger, he went on to give an account of his own observations concerning the disease. In the first place he said that the periphery of the colonies (Rasen) was not always so uniform as Harz had pictured it ; frequently single club-shaped cells projected widely beyond the margin. Here and there single, fine, pale, non-septate mycelial threads of scarcely measurable diameter appeared to bud outwards from the depth of the colony. Other colonies, however, were composed mainly of mycelial and hyphæ filaments, almost always the outer end of the latter carried a fine, dull, pear-shaped swelling. Between these there lay further larger pear-shaped or club-shaped cells, which at other places in the same colony seemed to be the only elements present. Lastly, there were present single quite small colonies composed entirely of fine mycelial threads. In addition to the large pear-shaped and club-shaped forms lying close together at the surface of the colonies, there were found the micrococcus-like, very small, round bodies mentioned by Israel. The centre presented an extraordinarily fine, not very abundant mycelium. From this there radiated in all directions numerous hyphæ, the ends of which became converted into the longish, pear-shaped or conidia-like structures. [The word conidia here used had previously been employed by Harz

* The italics were not in the original.

and by Israel.] On pressure the conidia were very easily detached from the hyphæ, but he was not able to observe any septa at the places where the clubs became detached.

Accompanying Johne's article were two plates containing numerous uncoloured drawings, nearly all of them representing single or banana-like groups of clubs, but also showing several separate colonies; most of the separate clubs were shown under very high magnification, and, with one or two exceptions, the objects depicted might have been obtained from colonies of the actinobacillus.

Dealing next with actinomycosis of the tongue, Johne went on to state that, among the museum specimens in the Veterinary School at Dresden, there were two preparations of chronic induration of the tongue or chronic interstitial glossitis, and only in one of these could the alterations described by Bollinger be found, whereas the other showed no trace of a mycotic infection beyond the signs of a chronic interstitial inflammation.

Wolff and Israel[4] were the first (1891) to succeed in cultivating the organism which is now generally accepted as the cause of actinomycosis.

Material for the cultures was obtained from two cases of human actinomycosis. The first was specially suitable for the purpose since it was a closed retro-maxillary tumour covered by intact skin. In the second case conditions were less favourable because it was one of open actinomycosis—primary actinomycosis of the lung with extension to the right breast, and breaking through the skin at many places.

The culture experiments were carried out as follows :—

The actinomyces granules were taken with every precaution and transferred to agar-agar, and also to hens' eggs. The agar tubes were placed under anærobic conditions, partly by Buchner's method and partly with nutrient media containing 1 per cent. of sodium formate. The best primary growths were obtained in those cases in which the material used for seeding had been well rubbed up. Incubation was at 36·5° to 37° C., and in subcultures colonies were visible with a hand lens in two or three days. The separate colonies were rounded, but not always quite round, and in some cultures they reached a size varying from a pin's head to a millet seed, and rarely the size of a lentil. In stab culture also the growth was in the form of colonies, and it occurred also along the needle track, and sometimes at the upper end of the stab. The colonies developing along the needle track remained small. After succeeding in cultivating the organism under the above anærobic conditions, a new series of attempts was made to obtain growth on agar containing 1 per cent of sodium formate. In stab cultures the growth was quite the same as under the previous anærobic conditions, but the surface growths were not so good as with the other method in which pyrogallol was used. Afterwards cultures were made under ærobic conditions at 37° C.; these were started from a primary anærobic culture. It was found that the results in the case of surface growths were not specially favourable, and in many cases no growth whatever developed on the surface of the medium. It was noticed, however, that a considerable amount of growth, in the form of characteristic colonies, occurred under the surface of the condensation water and on the surface of the medium covered by this liquid.

Repeated simultaneous cultures were made from the same culture as surface and stab cultures under ærobic conditions, and with surface cultures under anærobic conditions. The inferiority of the results of ærobic over anærobic surface cultures was pronounced, but there was little difference in the case of stab cultures. As a fluid medium an alkaline broth was used, sometimes in test tubes, but generally in Erlenmeyer flasks, the liquid being about 1 cm. in depth. The tubes or flasks were placed partly under ærobic and partly under anærobic conditions. The seed material was taken from abundant anærobic surface growths on agar, and the inoculation was in some cases made by using a platinum needle to deposit part of the surface and leave it floating on the broth. In other cases the seed material was distributed throughout the broth as finely as possible. There did not appear to be much difference in the result. Small white points of growth were visible in three to five days, partly floating in the broth and partly lying at the bottom of the flask. The anærobic conditions were somewhat better than the ærobic. The broth remained quite clear. No growth was obtained in nutrient gelatin in test tubes incubated at 16°-20° C. No growths at the same temperature were obtained on the surface of nutrient agar or in stab cultures. Repeated control experiments at summer temperatures and at 37° C. were made, the latter always developing slowly, whereas no growth occurred in those at room temperature after four or five weeks.

The results were summed up as follows :—

The actinomyces has a pronounced tendency to colony formation. The colonies seldom appear before the third, fourth, or fifth day. Growth is generally rather slow, and even when the colonies are close together they remain isolated in many cases for weeks or months. The larger colonies have not only a very remarkable growth on the surface, but also push very characteristic rootlets into the substance of the medium. The surface cultures are often—especially when thin—strikingly dry, and adhere firmly to the medium. The larger and older colonies are not seldom slightly yellow in colour. The most favourable temperature for growth is from 35°-37° C. Growth of the actinomyces is independent of the presence of oxygen as is proved by the good growths obtained under anærobic conditions, but the actinomyces is certainly no strict anærobe. In culture it retains its vitality for a long time. Successful sub-cultures were started from cultures nine months old.

In the growths the organisms occurred in the form of shorter or longer rods, or continuous or segmented threads, which have a straight or wavy course, or are distinctly screw-like ; coccus-like elements are also present.

The question remained whether all these different forms of growth belonged to one and the same individual fungus, namely, the actinomyces, and what was the genetic relationship of the one to the other. To decide this question hundreds of agar cultures were examined. What was found to be the simplest and most frequent form was uniform, generally straight, but often comma-like or more strongly curved organisms. The length and breadth of the rods was not always the same.

In 1902 Lignières and Spitz[5], in a work extending to 105 pages, described a disease which in the immediately preceding years had caused most serious losses among cattle in the Argentine Republic, and they named the organism which they found in the lesions the *Actinobacillus*. For the purpose of the present article it is not necessary to set out in detail the evidence marshalled to prove that

this was the actual cause of the disease and that it was morphologically, culturally and tinctorially different from the *Actinomyces* of Wolff and Israel. The two diseases had very similar lesions, but the two organisms had hardly a point in common except that they both tended to build up in the tissues where they were growing compact rounded masses in which the surface elements had the form of clubs. Whereas the main vegetative elements in the actinomyces were wavy, branched Gram-positive filaments, the organisms which formed the core of the granules in the new disease were minute, unbranched Gram-negative bacilli. The former were preferably anærobic, the latter frankly ærobic.

The common sites of the disease were the tongue, the submaxillary and pharyngeal lymphatic glands, and the jaw bones.

The illustrations of the actinobacillus in the work in question were of a very high character, and left it impossible for any competent person afterwards to experience difficulty in distinguishing between the actinomyces and the actinobacillus. At least, so one would have thought.

Homer Wright[6] has been highly commended for his researches regarding actinomycosis, the results of which he published in 1905. His material was derived from 13 human and two bovine cases. The latter did not include an example of the disease in the tongue, one being a diseased jaw and the other an omentum.

Summing up the results of his own researches and a study of the literature on the subject, he expressed the opinion that the only characteristic infectious agent in typical actinomycosis was the one described in his essay, which agreed in all important respects with that of Israel and Wolff. Between *Actinomyces* from the human and the bovine cases he " found no difference sufficient to justify their being classified as a separate species." That was not surprising, since, as already stated, his list of cases did not include a diseased ox tongue and his directions to other workers were as follows :—

" If one is working with a bovine case it is well to examine microscopically the disintegrated material, after mixing it with a drop or two of bouillon, under a cover glass, to see if filamentous masses are present. If they are not, or if they are few, proceed no further, but begin again with another granule, because the granules in bovine lesions sometimes contain no living filaments at all, but may be composed entirely of degenerated structures from which no growth of the micro-organism can be expected."

It is obvious that if Lignières and Spitz had acted in this way the actinobacillus might not yet have been discovered. In this connection it must be remembered that Wright was acquainted with the work of these two authors, which had been published three years previously, and he actually went so far as to say that he thought their observations required further confirmation, in spite of the fact that he knew they had been confirmed, without reserve, by Nocard.

In an article by Dr. M. Schlegel[7] which deals with actinomycosis in the second edition (1913) of Kolle and Wassermann's *Handbuch der Pathogenen Microorganismen*, there is a coloured illustration entitled "Section through a lentil-sized actinomyces nodule in an ox tongue." It is impossible to doubt that what is shown in that illustration is not the true actinomyces, but a conglomerate growth of the actinobacillus.

That figure has not been reproduced in the article on actinomycosis by the same author in the third edition (1928), whereas an excellent illustration of a genuine actinomyces colony from an actinomycoma of the jaw of an ox which appeared in the previous edition is retained.

In the second edition (1913) the account of actinobacillosis extends to less than one page. What is there said is a very condensed abstract of the monograph by Lignières and Spitz (published in 1902) regarding the disease in Argentina, and it contains nothing to suggest that the disease occurs in Europe.

In the third edition the same author expressed the opinion that further investigation was necessary to show whether actinobacillosis was actually different from actinomycosis, or whether the apparent differences were only due to the methods of staining !

At this point it may be affirmed with confidence that prior to the discovery of the actinobacillus two ætiologically distinct diseases of cattle were confounded under the term ætinomycosis, and it may be suggested as not improbable that Bollinger, Harz and Johne had examples of both diseases before them and failed to recognise the difference. The evidence in favour of the latter view will now be stated.

In considering the possibility of such a mistake it has to be kept in mind that the bacteriological methods available for the identification of bacteria in diseased tissues were incomparably less trustworthy in 1877 than they are to-day, owing to the later development of numerous refinements in fixation, embedding, section cutting, and staining, and it has also to be remembered that at the date mentioned it had not been found possible to obtain artificial cultures of the new organism. Furthermore, assuming for the moment that the diseased tongues examined by Bollinger and Johne were in reality cases of actinobacillosis, in view of the similarity of the granules in the tongues and the jaw bones it was natural to fall into the error of supposing that they were identical.

Lastly, and perhaps most convincing of all, it appears that no modern observer has been able to demonstrate the organism of Israel and Wolff in a case of wooden-tongue. F. Griffith[8] and Bosworth[9] in this country, and Hulphers and Magnusson in Sweden[10], failed to find it (in a total of 55 unselected cases), but constantly found the actinobacillus, which they also found most frequently in the diseased submaxillary and pharyngeal lymphatic glands. And here it must be noted that there is no room for any differences of opinion regarding

the ætiological rôle of the two organisms in cases affecting the bones of the head in the ox, since both the actinobacillus and the actinomyces of Israel and Wolff have repeatedly been found in these lesions.

It might have been thought that Harz's account of the specimens which he examined must make it possible to decide confidently whether the granules on which he fixed the botanical position of the " new fungus " belonged to the actinomyces or the actinobacillus, but that is not the case.

The first few pages of the article by Harz ([11]) are devoted to an historical review of the lower fungi, including the saccharomyces the ascomycetes, and the moulds. He then continues :

" In 1870 Prof. Hahn discovered in a peculiar diseased ox tongue (so-called wooden-tongue) characteristic organised structures which he, at the time, designated as possibly a species of penicillium, but without following the interesting case further.

" Meanwhile, other specimens of wooden-tongue were sent to the Veterinary School, and Prof. Bollinger, who investigated the disease, was kind enough to provide me with material for examination with regard to the botanical position of this questionable organism."

Harz here quotes from Bollinger's description of the disease (see pp. 93 to 95), and this part contains the sentence : " Similar appearances are presented by the gums and the tissue of the tongue."

Proceeding to give an accurate description of the fungus, he gives a reference to the lecture which he had delivered on May 16th, 1877, at a meeting of the *Gesellsch. f. Morph. u. Physiol*, in Munich. He than proceeds with the description as follows :—

" The fungus appears in the form of pale yellow, rounded, gland-like (drüsenformige) growths, which, when slightly magnified, show a thready (faserige) substance radiating from the centre to the periphery ; they recall the appearance of the sphæro crystals of inulin, fatty-acid crystals, &c. They may reach the size of 0·11 mm. As a rule two or more of them are united like a mulberry, which, from the smallest beginning, may reach over 1 mm. in diameter ; therefore, they are frequently easily recognised with the naked eye as pus-coloured granules of the size of millet seeds, which, when the tissue is cut, can easily be squeezed out with slight pressure. Sometimes they show lime crystals on their periphery and in their interior."

" With stronger magnification one recognises distinctly and easily that these rounded, thready, radiating bodies are composed of cells which possess a thin membrane and homogeneous weakly refractile contents. Iodine, aniline, and picric acid produce the known characteristic appearances. The cell membrane does not show the blue cellulose reaction, and it is indifferent to polarised light. Alcohol, ether, acids, and alkalies have no recognisable effect on them.

" Under slight pressure the round growths of the fungus fall into numerous, unequal sized, wedge-shaped pieces, corresponding to a number of individuals, which according to the age, favourable or unfavourable development, may have a size of from 10-15 or 50 mikr. with equal or greater breadth at their ends."

"The pointed end of this wedge represents the oldest part of the individual fungus present in the centre of the granule (Pilzkugel) whereas the opposite

peripheral part of the wedge is formed by the youngest cell generation. With accurate examination (Hartn. System imm. No. 9) one recognises that each single individual begins with a conical, ·05 mikr. broad to 4·5 mikr. long basal cell representing the mycelium. This next develops two to nine or more short segmented hyphæ, which branch in a somewhat irregular manner, and so ultimately run out into a great number of terminal branches, all ultimately ending in a slightly convex meniscus representing a sort of gland or tuft."

" The diameter of the hyphæ is very unequal, varying from 0·5 to 2 mikr. Septa are distinct in a few, but generally not clearly recognisable, so that one is frequently inclined to believe that the majority of the branches of the hyphæ are directly connected with the main branches of the whole individual, and that all the branchings are nothing more than outgrowths (ausstulpungen) and constituents of a single cell. Iodine, aniline and potash, and various other reagents often enable one to recognise the presence of septa (Scheidewande). The contents of the hyphæ and hyphæ cells mostly appear homogeneous, but sometimes finely granular, or provided with small vacuoles which are light grey, weak yellow, or almost colourless. On the terminal branches of the hyphæ are found the multiplying cells (conidia), partly single, partly two or three together, or, not rarely, two or three united to form a chain. They are somewhat variable in respect of size and shape, like an inverted egg, oval, round or elongated, thus as polymorphic as the hyphæ, commonly blunt and broadened at their upper ends, pointed at their lower ends, and very often running out into short, thin stalks, 4-12 mikr. long by 1·5-4 mikr. broad, single celled, with a thickish membrane, and weakly refringent apparently homogeneous contents. They stain like the hyphæ, but mostly somewhat darker. In very occasional cases I believed I could detect protoplasmic particles and clumps in their interior."

From what he had seen, and from the study of a great number of preparations of the fungus found in the diseased tissue, he gave the following as his idea of the method of development.

" The conidia (clubs) begin first at several points of their broad ends, as also sometimes later in the middle and further downwards, to bud outwards, whereas the lower, thin, and mostly pointed end, representing the mycelium, elongates slightly and then undergoes no further change. The budding takes place quite in the manner of the common yeasts, only that the number of the young buds is greater and no separation occurs ; I might compare them in a certain degree to the budding of a mucor-hefe-colony. These grow out to cylindrical tubes, which then repeat the process at their anterior ends, until after from four to eight repetitions the fungus has reached its normal size. In most cases the basal parts of the newly developed cell remain sterile, the upper ends again forming buds. In the same way, after apical budding, the cells appear to lose the power to produce new cellular individuals. Ultimately, as in many of the moulds, the conidia bud out from the terminal branches of the hyphæ. The partition walls, as already stated, are indistinct in the hyphæ. It is only in the conidia that they are pretty sharply marked off.

" Some time after the ripening of the conidia, the old hyphæ appear to die, as is the rule in the majority of the mould fungi, such as penicillium, &c., but there remains the possibility that single, old, mycelial cells, or the undermost hyphæ cells, may retain the power to form new buds under certain favourable conditions. They fall, eventually, into a finely granular mass and disappear.

" Afterwards, from one conidium an individual is formed with many new conidia, the latter bud again in the same way, and there is thus formed a larger colony of the more or less mulberry appearance. These colonies also produce new conidia at their surface, and from these ultimately arise larger masses, which (after disappearance of the older fungus growth) often have a large cavity in their interior.

" Besides the above described ordinary form of this parasite, there occur in the jaw bones of animals affected with this mycosis still other granules, which consist of more delicate but proportionately longer hyphæ, and which, at their ends, have conidia of scarcely half the size. Others, however, show the same sort of branching formation of new conidia by constriction, and other morphological peculiarities. I believe these to be imperfectly developed (*verkummerte*) forms of the same fungus."

In the text of the article by Harz there is an illustration containing 20 figures (see page 103), which are all obviously more or less diagrammatic and represent a magnification far in excess of the one mentioned in the text. They enable one, however, to follow better what Harz believed to be the formation of a *Pilz individuum*.

Very unfortunately, it is not possible to infer whether he had before him colonies corresponding to the *Actinomyces* of Wolff-Israel, or colonies of the *Actinobacillus* of Lignières and Spitz.

The text suggests that the greater part of the description of the parasite, occupying four pages, applies to granules obtained from a tongue, or tongues, and it will be observed that the last paragraph quoted refers to the granules found in diseased jaw bones. In that paragraph there is a reference to Fig. 20 in the Plate, which is more suggestive of a colony of the Wolff-Israel type than any of the other 19 figures, but could hardly with certainty be identified as such.

Since Harz had before him diseased tongues as well as diseased jawbones, and since it is extremely probable that the tongue disease was actinobacillosis, it may never be possible to decide which of the two organisms has, by priority, the right to the designation *actinomyces*.

A point possessing some interest is the question whether the actinomycosis and actinobacillosis are equally amenable to treatment with potassium iodide.

Although it appears that this treatment had previously been practised by some veterinary surgeons on the Continent it was Thomassen's article[9] (1885) that led to its wide adoption in cases of wooden-tongue. In that article, after describing the clinical characters of actinomycosis in cattle, he dealt with its treatment as follows :—*

" In the cases where the bones of the head are attacked no treatment has hitherto given good results. The tumours in the soft parts may be treated with success. In the cases in which they are circumscribed, and not situated too deeply, excision is the infallible remedy.

" In the cases in which this radical therapy cannot be employed, the

* In the course of the article Thomassen mentions that in one of his cases (in 1879), affecting the frontal and maxillary sinuses and the nasal cavity, Professor Pekelharing found masses of the actinomyces in the lesions.

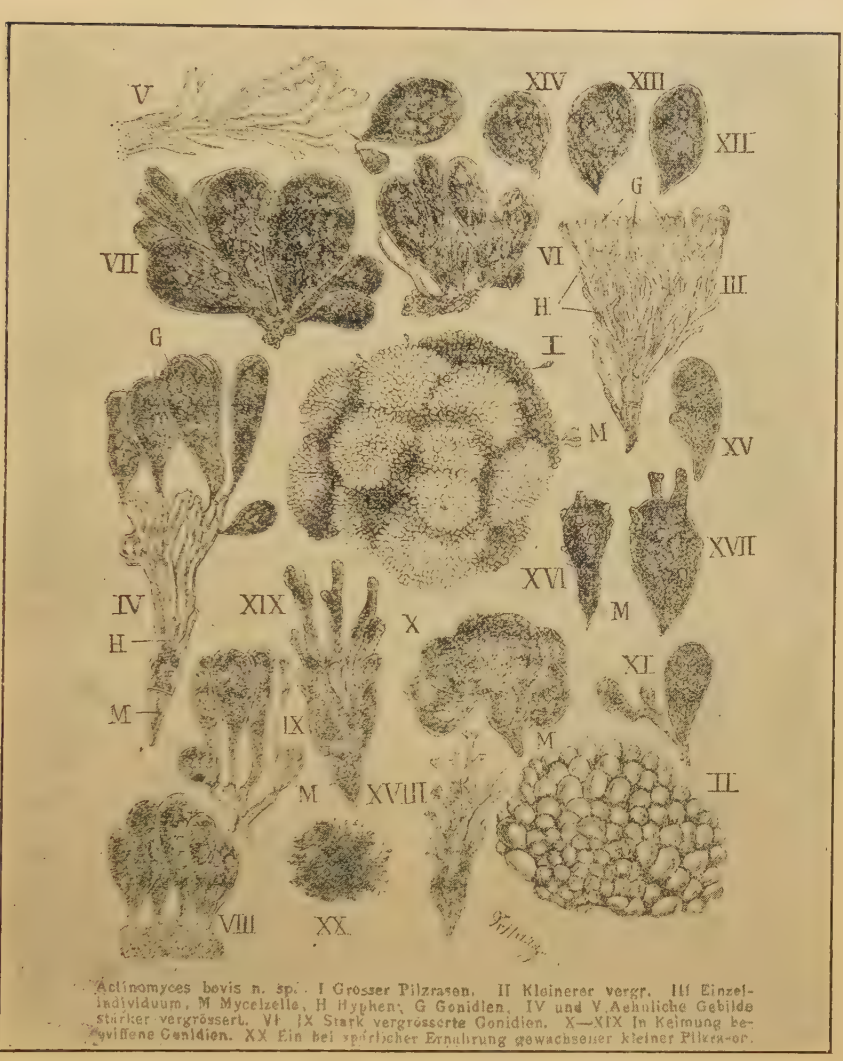

Plate that accompanied the article by Harz.

following treatment has, on many occasions, given excellent results in my hands :—

" Make a preliminary puncture with the actual cautery and then enlarge the opening with a bistoury. This is especially indicated in the case of a tumour with walls attaining a thickness of several centimetres.

" After having evacuated the contents, one introduces tow saturated with egyptiac ointment. This dressing is repeated daily. Ordinarily the greater part has disappeared after about 15 days, leaving only a slight cicatrix with retraction of the skin. Other caustics and disinfectants might replace the egyptiac ointment.

" It is especially on the treatment of the glossitis, hitherto considered by the majority of practitioners as incurable, that I wish to fix the attention of practitioners. It consists in the administration, in the form of a drench,

of 8 to 10 grams of iodide of potassium daily. Along with this internal treatment one may apply tincture of iodine to the tongue after having scarified this organ, especially at the places where one can discover the before mentioned nodules.

" The first treatment, continued during 15 days, generally gives a satisfactory result. The tongue soon re-acquires its normal suppleness, and the patient is able to take some food. After treatment for five or six days, it is always advisable to interrupt the administration of the iodide of potassium for two days.

" Probably we have here to do with an antiseptic and atrophic (atrophoiante) action of free iodine after decomposition of the salt in the organism, which procures a truly astonishing success in some days.

" Sometimes one observes during the treatment a slight irritation of the mucous membrane of the respiratory passages or conjunctiva, even a slight exanthema of the skin. It is then a case of the irritant action of the free iodine after secretion by these ways of iodide of sodium, formed in the circulating blood."

Ample evidence in favour of iodide of potassium in the treatment of the lingual disease has been furnished from all parts of the world, but it must not be overlooked that Thomassen found it unsuccessful in the osteo-sarcoma form. Apparently the treatment has been much less successful in human cases, and a possible explanation is that the drug is ineffectual against the actinomyces of Wolff and Israel, which is responsible for the disease in man. Referring to the treatment of actinomycosis in the human subject, Colebrook[13] stated that, on enquiring into the origin and the general experience of the iodide of potassium treatment he failed to find that it had any assured scientific basis, or even a sufficient vindication by results. Most of his cases had been treated by potassium iodide and by incisions before they came to the Inoculation Department of St. Mary's Hospital, but the iodide had not usually been pushed beyond 30-40 gr. per diam. ; in one or two instances 60-90 gr. had been given. In these doses the drug had not exercised any appreciable effect upon the disease. That, however, would not explain a general failure of the treatment of the disease affecting the bones of the head in cattle, since it is now known that both actinomycosis and actinobacillosis occur in that position.

It is possible, however, that it is the nature of the tissue that affects the result, for practically all bacterial infections tend to be less amenable to treatment where they involve osseous tissue.

The matter deserves further consideration, and when in future the iodide of potassium treatment is tried in cases affecting the jaws, it should be determined in advance whether the disease is actinomycosis or actinobacillosis.

To associate under the same name diseases that are fundamentally different, and similar only in non-essential characters, can only serve to hinder progress in clinical diagnosis.

Actinomycosis and actinobacillosis are as distinct as tuberculosis and glanders, and are similar only in respect of their common sites and the gross appearance of their lesions. Even in that way they are different,

for the tongue is the commonest site in the one and the jaw bones in the other. It is quite possible, however, that they are distinguishable clinically, but any such differences are not likely to be discovered if veterinary surgeons are encouraged to be content with recognising that any given case is either one or the other. Increased skill in clinical diagnosis can only be acquired by the practitioner who knows exactly what he is dealing with, and identification of the organism is at present essential for that, except perhaps when the affected part is the tongue, since it appears probable that there are few or no cases of actinomycosis in that organ.

In the case of chronic staphylococcus infections, the argument against inclusion under the name actinomycosis is still stronger.

Conclusions.

1. Actinomycosis and actinobacillosis are ætiologically distinct diseases, and they should no longer be united under a common name.

2. Actinomycosis is the disease caused by the *Actinomyces bovis* which was first cultivated by Wolff and Israel. It includes practically all the cases hitherto so named in man and a minority of the cases in cattle in which the bones of the head are affected.

3. Actinobacillosis is the disease caused by the *Actinobacillus lignieresi*, which was first cultivated and accurately described by Lignières and Spitz. In cattle it apparently includes all cases of the so-called " wooden-tongue " in all parts of the world, and an uncertain proportion of cases involving other parts of the body, including the bones of the head.

4. It appears to be possible that Bollinger and Harz in 1877 examined cases of both diseases but failed to observe the differences in the causal organisms.

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STUDIES IN LOUPING-ILL.

(An Encephalomyelitis of Sheep.)

I.

Section A : A Note on the Infectivity of Blood.

Section B : A Field Experiment (1931), with a preliminary note on the Nature of Tick-borne Fever.

W. S. Gordon,
A. Brownlee,
D. R. Wilson,
and
J. MacLeod.

From the Moredun Institute,
Animal Diseases Research Association,
Edinburgh.

Introduction.

By louping-ill in sheep is currently understood a disease characterised by cerebellar ataxia and disorder of brain functions, lasting from about a day in acute cases to some weeks in lingering chronic cases. The mortality is high, but *animals which recover do not usually develop another attack.* The association of the disease with particular pastures is well established and sheep brought from a locality in which louping-ill does not occur into a louping-ill district are liable to develop the disease in a large proportion of cases.

In the following paper the evidence will be reviewed showing that, (1) louping-ill is due to a filtrable virus which is communicable to mice as well as sheep ; (2) the virus may be present in an infected sheep without producing typical louping-ill, but blood drawn during the febrile reaction which accompanies this infection contains the virus ; (3) such atypical infections may be merely a febrile reaction or it may be a cause of sudden death in sheep ; (4) under natural conditions louping-ill is probably tick borne ; (5) recovery from infection, either naturally or experimentally produced, results in immunity ; (6) the investigation of louping-ill is rendered difficult by the fact that a distinct type of infection, " tick-borne fever," may co-exist with it. " Tick-borne fever " is clinically and immunologically distinguishable from louping-ill infection, but it probably aggravates the harmful effects of the latter.

The Infective Agent.

A systematic investigation of louping-ill was commenced in 1929 at the Moredun Institute, Animal Diseases Research Association, Edinburgh, and Pool, Brownlee and Wilson (1930), who carried out the investigation, recorded *inter alia* the successful transmission

of the disease in series from sheep to sheep with material obtained from the central nervous system of two sheep affected with louping-ill. No bacterial agent capable of causing the disease was demonstrated, nor did the authors arrive at a definite conclusion as to the nature of the infective agent ; they stated, however :—" While filtration experiments have given inconclusive results, none of the work carried out has produced evidence against the existence of an ultra-microscopic virus . . ." and further :—" The type of infection, coupled with the circumstantial evidence that in natural circumstances it is transmitted by ticks, suggests the possibility that it may be associated with Rickettsia."

In continuation of the work of Pool, Brownlee and Wilson, Greig, Brownlee, Wilson and Gordon (1931) repeated the transmission experiments in sheep, established that the infective agent was a filtrable virus, and that it was present in the blood during the febrile reaction. They also showed that sheep which had recovered from the thermal reaction which follows inoculation of virus were immune to subsequent intracerebral inoculation of infective material.

Further progress was facilitated by the observation of Alston and Gibson (1931) that sterile filtrates of material containing the virus of louping-ill reproduced the disease on intracerebral inoculation into mice, and also that the disease could then be transmitted from mouse to mouse indefinitely. The work of Alston and Gibson has been amply confirmed by Czarkowska-Gladney and Hurst (1931) and also in our own experiments, in which several thousand mice have been used.

The symptoms exhibited in the experimentally induced disease in the sheep and mouse indicate involvement of the central nervous system. In the sheep the symptoms are essentially those of a cerebellar ataxia ; in the mouse paralysis of one or more limbs usually develops, indicating involvement of the spinal cord.

Brownlee and Wilson (1932) have described the histo-pathology of the naturally occurring disease in the sheep and of the experimentally produced disease in the sheep, mouse and pig. They showed that in the sheep some degree of destruction of the Purkinje cells of the cerebellum occurs, while in the mouse there is necrosis of motor nerve cells of the spinal cord. It was also shown by Hurst (1931) that " . . . intracerebral inoculation of the virus of ' louping-ill ' is productive in both the mouse and the monkey of a definite encephalomyelitis which, while partaking of the general characteristics of neurotropic infections, possesses features peculiar to itself."

The Association of the Tick (Ixodes ricinus) with Louping-Ill.

Circumstantial evidence favours the view that the British tick, *Ixodes rincinus*, *L.* plays an important part in the transmission of louping-ill. Stockman (1918), after completing transmission experiments with ticks, concluded that the highly febrile and sometimes fatal disease induced by the infestation of normal sheep with ticks

which had in their previous stage engorged on affected sheep, was in fact louping-ill. Undoubtedly he produced reactions and sometimes death but the nature of these reactions is a matter for further discussion.

In the paper by Greig *et al.* (1931) experiments designed to test the transmissibility of louping-ill virus by infestation of sheep with ticks are described. In one of the experiments quoted two normal sheep were infested with numerous nymphal and larval ticks gathered from the pastures of a diseased farm during the louping-ill season. Neither of these sheep developed characteristic louping-ill, but on the fourth day after infestation both developed thermal reactions. From one of these sheep blood was drawn during the febrile phase and passed in sheep through two generations by subcutaneous injection; louping-ill did not develop in the inoculated sheep, but a thermal reaction occurred on the fourth day. After recovery from this thermal reaction the sheep were tested for immunity to louping-ill, and all developed a temperature reaction which was followed by typical symptoms of the disease. In discussing these infestation experiments the authors concluded that the first thermal reaction was not due to infection with the virus of louping-ill, as after recovery the sheep were not immune to inoculation with this virus.

There is thus evidence that two sets of workers obtained reactions in sheep by infesting them with ticks collected either from sheep affected with louping-ill (Stockman, 1918), or with ticks collected from diseased farms during the louping-ill season (Greig *et al.*, 1931). While Stockman regarded the reactions as representing louping-ill, Greig *et al.*, in the light of their later acquired knowledge, were able to determine that the febrile affection obtained by them was immunologically distinct from louping-ill.

Immunity.

It is well known that sheep on a diseased farm acquire an immunity to louping-ill, and that it is a highly dangerous practice to introduce sheep from a healthy farm; that sheep on a diseased farm actually acquire immunity has been demonstrated by Pool, Brownlee and Wilson (1930), who inoculated 82 such sheep with louping-ill virus and found that 16 of them were definitely immune.

Immunity can also be induced artificially. Greig *et al.* (1931) found that the introduction of living virus to sheep by intranasal insufflation, intradermal inoculation, or subcutaneous injection, was followed by a thermal reaction and that sheep so treated were immune to subsequent cerebral inoculation.

From a consideration of the literature published up to the present time the following may be concluded :—

(1) The infective agent of louping-ill is a filtrable virus possessing neurotropic characters.

(2) The essential pathology of the disease caused by this virus is represented by a meningo-encephalo-myelitis.

(3) The mouse is susceptible to infection by intracerebral inoculation, and is a suitable test animal for the detection of virus in any given tissue.

(4) Virus can be preserved for long periods in infective sheep brain which has been dried and powdered.

(5) In the sheep a thermal reaction to the introduction of virus is followed by immunity to subsequent intracerebral inoculation of virulent material.

(6) Ticks collected from louping-ill pastures are capable of producing a temperature reaction when allowed to feed on normal sheep. By means of immunity tests this reaction has been proved to be the manifestation of a condition other than that of louping-ill.

(7) Blood drawn during the febrile reaction from a sheep experimentally infected with louping-ill contains the virus.

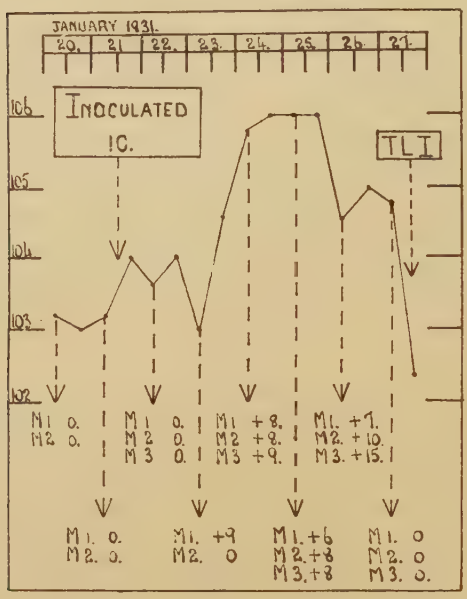
Section A.

A Note on the Presence of Virus in the Blood of Infected Sheep.

It has already been demonstrated that the blood of sheep affected with louping-ill may contain living virus (Pool, Brownlee and Wilson, 1930, and Greig *et al.*, 1931). In view of some contemplated experiments with reference to tick transmission of louping-ill, it was decided to obtain an accurate knowledge of the conditions under which virus could be recovered from the blood, and so ascertain when ticks in their various stages of development would suck virus-containing blood from an infected sheep. Our experiments have been made with blood from sheep infected by intracerebral or subcutaneous injection of virulent virus-containing sheep brain, and in some cases with Berkefeld filtrates of such material. Following this injection, samples of blood were collected daily from the jugular vein into sterile tubes containing sufficient potassium citrate to prevent clotting, and mice were used for demonstration of the presence or absence of virus. The citrated blood was diluted to one in five or one in ten with normal saline and 0·1 c.c. of this injected intracerebrally. Inoculated mice were kept under observation for a period of not less than 20 days before a negative result was accepted. We have used a number of sheep for these experiments, and Chart I records in full the result of one such experiment. In this case the sheep was inoculated intracerebrally with a Berkefeld filtrate. A temperature reaction commenced on the second day ; on the fourth day the sheep was dull and showed progressive symptoms of inco-ordination in gait, and by the sixth day the body was in a state of generalised tremor. At this stage the animal was unable to rise and was destroyed. Blood was drawn daily, as indicated by the arrows leaving the underside of the curve, and mice were inoculated intracerebrally with 0·1 c.c. of blood diluted one in ten with saline.

CHART I.

TEMPERATURE CHART OF A SHEEP AFTER INTRACEREBRAL INOCULATION WITH THE VIRUS OF LOUPING-ILL.



M.=Mouse.
O.="No Take."
+X.=Length of Incubation in Days.
TLI. =Typical Louping-ill.

It will be observed that the virus was present in the blood during the course of the disease, appearing on the day on which the temperature rose and persisting until the occurrence of the rapid defervescence before death, at which stage no virus could be detected. In some of our experiments virus in low concentration was shown to be present in the blood up to the time of death of the sheep.

In sheep injected subcutaneously with virulent material there is usually a diphasic temperature curve, and we found on several occasions that during the period of remission between the two phases virus could not be demonstrated in the blood.

From the various experiments carried out, we conclude that louping-ill virus invades the blood stream concurrently with the initial rise in temperature and is present in demonstrable amount during the febrile stage of the infection, and that in most instances a fall in temperature is followed by the disappearance of much or all virus from the blood. These findings are of much value in tick transmission work, as we know now when to feed ticks on presumably infected sheep in order to be sure of their taking in virus.

Section B.

A Field Experiment (1931).

Early in 1931, the Director of the Institute, Dr. J. Russell Greig, arranged for a field experiment in which a group of sheep, rendered immune to louping-ill by the subcutaneous inoculation of living virus, was grazed with a similar number of susceptible sheep on the tick-infested ground of a louping-ill farm. The sheep were transferred early in April to graze on two infected pastures, A and B, each of about 35 acres. They were kept under observation during April, May and part of June—the season during which ticks are most active and during which louping-ill occurs in its maximal incidence. The temperatures of all the sheep on Pasture A were recorded daily, and 15 of the " immunes " and 15 " controls " were weighed twice weekly. The sheep on Pasture B were not handled, but were kept under close observation. It was anticipated that all the sheep would become infested with ticks from these pastures. The time and comparative degree of infestation was recorded by making a daily count of the fully fed nymphal ticks collected from the head, neck and ears of each sheep. This count included only one of the three stages in the life cycle of the tick, but it seemed sufficient to indicate the degree of infestation.

The test sheep were thus exposed to the chance of natural infection and the incidence of louping-ill was observed in each group. On the completion of the field experiment the surviving sheep were returned to the laboratories and were tested for any immunity acquired as a result of exposure to natural infection.

The history of the sheep exposed on Pasture A and those on Pasture B will be dealt with separately, and, in connection with these, Appendices A, B and C have been prepared to give a brief history of each sheep used in the experiment. Appendices A and B refer to the sheep grazed on Pastures A and B respectively, while Appendix C deals with the sheep used as " controls " in the various immunity tests.

Preliminary Experiments in the Immunising of the Sheep used in the Field Experiment.

It was shown by Greig *et al.* (1931) that the subcutaneous inoculation of sheep with living virus was followed by a thermal reaction, and that sheep so treated were immune on recovery. No evidence had been produced to show that any danger attended such a method of treating sheep. From our knowledge of the effect of subcutaneous injection of certain other viruses, we thought it wise to investigate the safety of this method of producing immunity, and to define the dose of living virus which when injected subcutaneously would produce no harmful results and would render the treated sheep immune. Accordingly, twelve sheep were selected for inoculation with living virus. The details of the experiment are shown in Table I.

TABLE I.

Sheep No.	Subcutaneous inoculation of louping-ill virus Dose 10 c.c. D.B. 5. Date, 11/2/31.		Intracerebral inoculation of louping-ill virus. Dose 1 c.c. 1/100 D.B. 5. Date, 4/3/31
	Dilution.	*Result.*	
994	1/10	Temperature reaction. Sheep slightly dull.	No reaction.
45	1/10	Temperature reaction.	Died at operation.
29	1/100	Temperature reaction.	No reaction.
*42	1/100	No temperature reaction.	No reaction.
996	1/1,000	Temperature reaction.	No reaction.
21	1/1,000	Temperature reaction	No reaction.
986	1/10,000	Temperature reaction. Sheep dull.	No reaction.
987	1/10,000	Temperature reaction.	No reaction.
989	1/100,000	Temperature reaction.	No temperature reaction. Sheep dull.
991	1/100,000	Temperature reaction.	Slight temperature reaction.
20	1/1,000,000	Slight irregularity of temperature.	Temperature reaction.
995	1/1,000,000	Slight irregularity of temperature.	Temperature reaction. T.L.I. March 10, destroyed.
67	—	—	Temperature reaction. T.L.I. March 11, destroyed.

T.L.I.=Typical louping-ill.
D.B.5.=Dried brain strain 1930-1a. (A dried and powdered brain from a louping-ill sheep.) Saline suspensions of this product were used as infective material.
*Sheep 42 was probably immune before treatment.

The result of this titration experiment further substantiated the previous findings that a sheep which gave a decided temperature response to subcutaneous inoculation of virus was solidly immune when subsequently tested by intracerebral inoculation. From this experiment the lowest dose of virus required for the production of solid immunity was taken to be 10 c.c. of 1 in 10,000 *Dried Brain* 5, but as this dose had been decided by the use of two animals only it was considered advisable to use a stronger suspension of virus, and 10 c.c. of a 1 in 1,000 suspension was injected to ensure that the sheep would be definitely immune to intracerebral inoculation of virus.

Sheep Grazed on Pasture A.

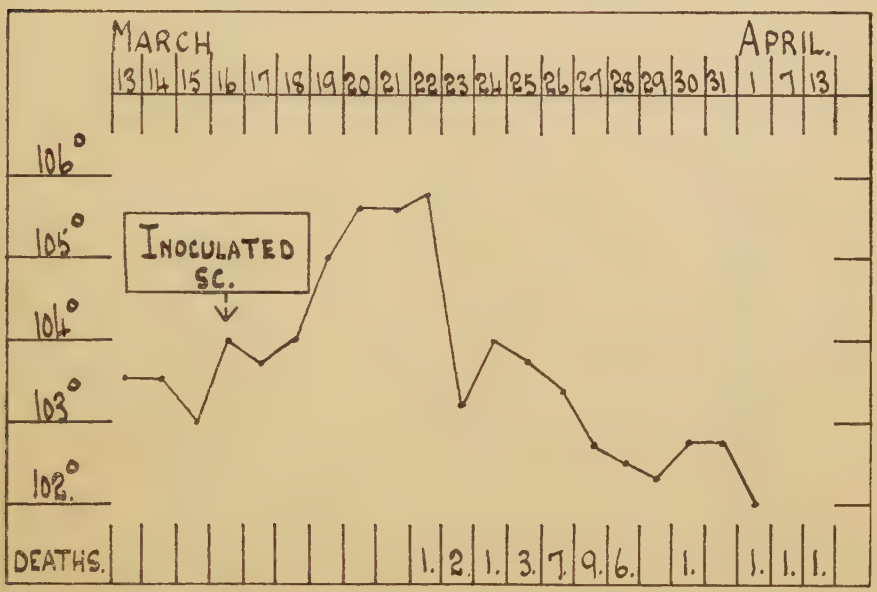
Immunisation.

Fifty sheep were injected on March 16th with 10 c.c. of a 1 in 1,000 saline suspension of *Dried Brain* 5. Their temperatures were recorded daily until April 2nd, when the survivors (with the exception of three—serial Nos. 25, 14 and 33) were transferred to experimental Pasture A. The average daily temperature of the sheep was calculated and from the figures thus obtained the curve in Chart II was prepared. (This chart is referred to as a " composite " temperature chart, and the average rise in temperature is spoken of as a " composite " rise. We have employed this expression in describing the

temperature history of each group of sheep used throughout the experiment.)

CHART II.

Composite Temperature Chart of the Reaction in 50 Sheep after Subcutaneous Inoculation with the Virus of Louping-ill.



Thirty-three of the 50 sheep died, and all types of cases developed, including sudden deaths without symptoms having been observed. From this experience it is obvious that living louping-ill virus is unsafe for immunising purposes, as a dose shown to be safe on a small number of sheep may cause disastrous results when used on a moderately large scale.

The 17 surviving sheep (*vide* Appendix A1, serial Nos. 2, 6, 7, 9, 12, 13, 16, 19, 26, 27, 29, 30, 31, 34, 47, 48 and 49), together with seven others (*vide* Appendix A1, serial Nos. 51 to 57), which were already known to be immune, making a total of 24 sheep, formed the protected group for this experiment.

Immediate Results of Exposure to Natural Infection on Pasture A (from April 2nd to June 15th).

To the above 24 immunised sheep were added 25 normal sheep believed to be susceptible to louping-ill as shown by the results of inoculating some of their fellows with living virus ; they comprised the control group (*vide* Appendix A2, serial Nos. 58 to 82).

The temperatures of both groups of sheep were recorded daily while they grazed on the infected pasture, and 15 of the protected sheep, with 15 controls, were weighed twice weekly. Charts III and IV illustrate this phase of the experiment.

CHART III.

COMPOSITE TEMPERATURE CHART OF "IMMUNES" AND "CONTROLS" DURING THE PERIOD OF EXPOSURE TO LOUPING-ILL INFECTION ON A "DISEASED FARM."

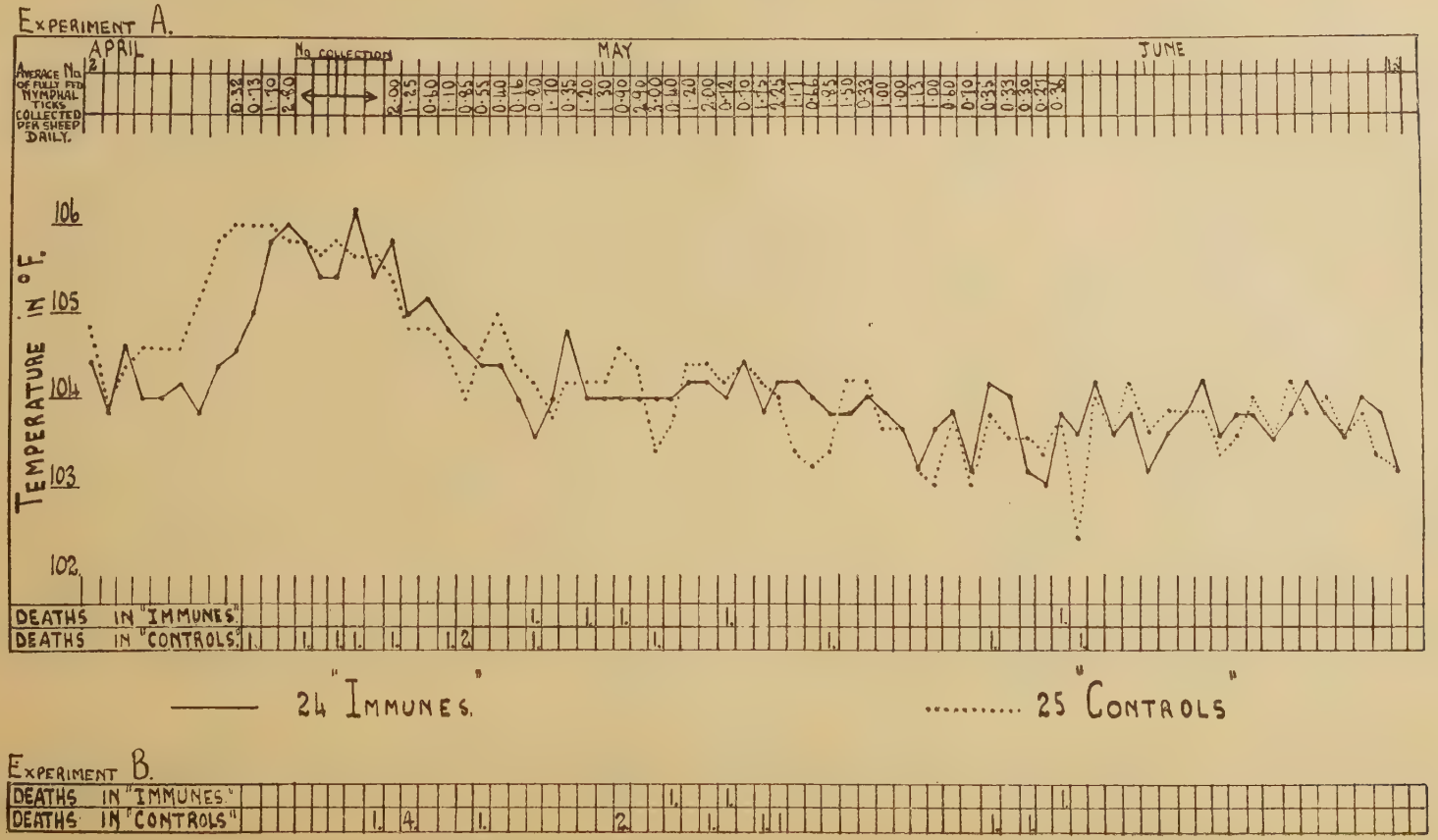
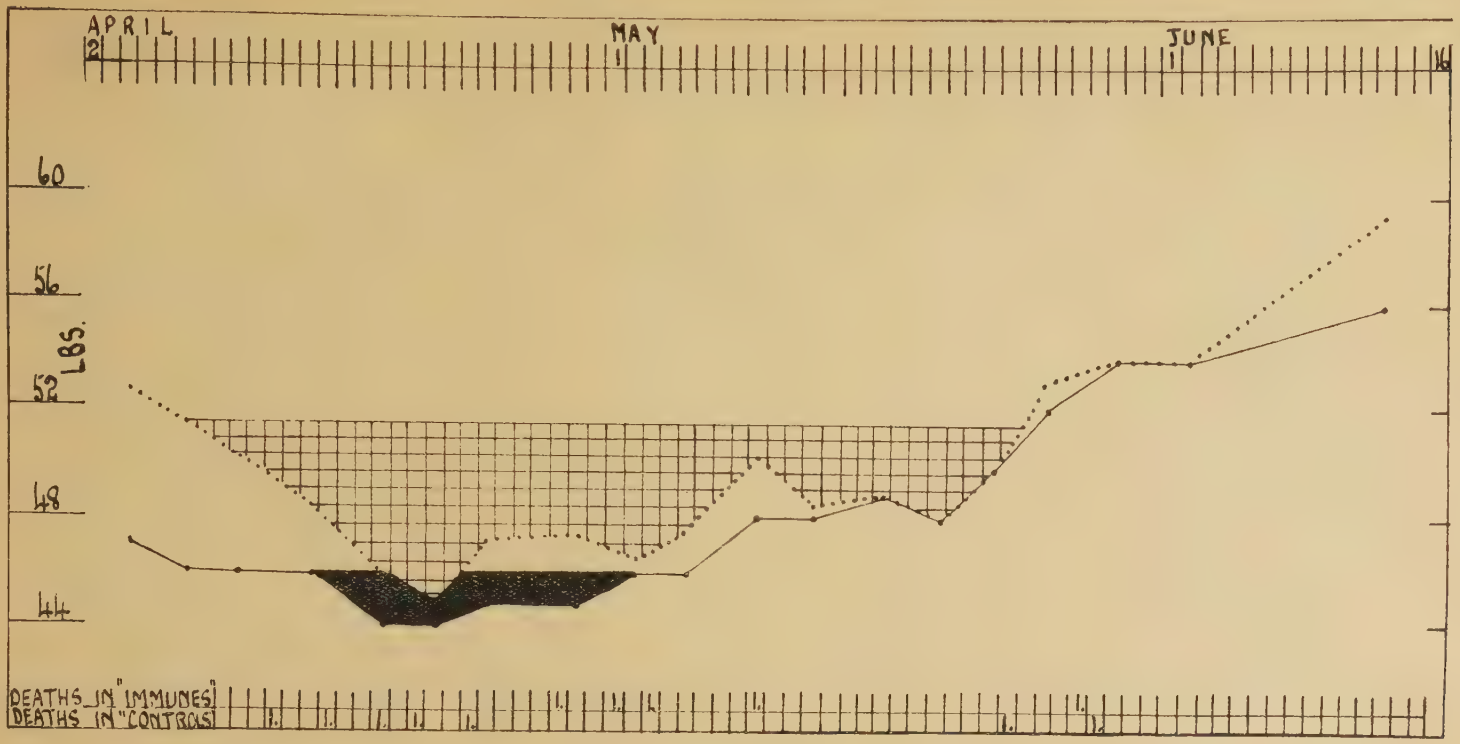


CHART IV.

COMPOSITE CHART OF THE WEIGHT IN LBS. OF 15 "IMMUNES" AND 15 "CONTROLS" DURING THE PERIOD OF EXPOSURE TO "LOUPING-ILL" INFECTION ON A "DISEASED FARM."



The following points are brought out by an examination of these charts :—

1. *Infestation of the Sheep with Ticks.*

The sheep became infested with ticks from the pasture, as indicated by the average daily collection per sheep of fully gorged nymphal ticks. Nymphs replete with blood were collected eight days after the sheep had been put to graze on the experimental pasture. If the " composite " rise in temperature which commenced on the fifth and sixth days was due to an infective agent or agents, transmitted by the tick, then this reaction was produced by a comparatively small number of ticks. It has been shown by one of us (J.M.) that the nymph requires from four to six days to fill itself to repletion with blood. This being so, the ticks collected between April 10th and 13th must have become attached to the sheep between April 4th and 7th. It is hardly conceivable that the light infestation, as judged by these collections, was capable of producing the " composite " rise in temperature by mechanical irritation alone. If the ticks were responsible, it seems feasible to suppose that they were transmitting an infective agent which found in the sheep a suitable host for growth and multiplication.

2. *A "Composite" Rise of Temperature in the "Immunes."*

On the seventh day a rise in temperature commenced in the 24 immunised sheep. By the eleventh day the average temperature was 106° F. and it remained high for eight days. Thereafter it fell gradually until the twenty-second day and remained comparatively steady from this time till the end of the experiment. This temperature reaction, if due to the virus of louping-ill, was quite at variance with our previous finding that after the sheep had given a thermal response to the injection of virus it was subsequently immune. It therefore seemed highly probable that this reaction was not due to the virus of louping-ill. We have since shown that this reaction is due to an infective agent, the nature of which is at present unknown, and which is transmitted by the tick. We have provisionally named this condition " tick-borne fever."

3. *A " Composite " Rise of Temperature in the "Controls."*

On the fifth day a rise in temperature commenced in the 25 control sheep. By the eighth day the average temperature was 106° F. and remained high for nine days. Thereafter it fell gradually until the twenty-first day. Fluctuations in the " composite " temperature occurred until about the forty-third day, and this is the approximate date on which the control sheep commenced to put on weight rapidly.

The foregoing results show that the reaction in the " controls " had a shorter period of incubation than that in the " immunes," which suggests a difference in the nature of the causal factor of the two reactions. As a result of more recent experiments we have

learned that the period of incubation which follows louping-ill infection is shorter than that which follows " tick-borne fever " infection. This knowledge suggests that the early febrile reactions which occurred in the control sheep after exposure to natural infection was caused by the virus of louping-ill, while in the " immune " sheep the longer incubation prior to the febrile reaction was probably a manifestation of " tick-borne fever " infection *per se*.

4. *The Mortality Incidence in the Immune Sheep.*

Five deaths occurred in the 24 immunised sheep during the experiment—a mortality of 20·8 per cent. This was not unexpected, since 17 of these sheep were in a debilitated convalescent state following immunisation, and this condition was further aggravated by the subsequent infection, " tick-borne fever," which was acquired on the experimental pasture. The five deaths were confined to this group of 17 sheep ; none of the seven healthy immune sheep died, although each developed a severe " tick-borne fever " reaction. (For the history of each sheep see Appendix A1 and A2.)

Material for diagnosis was obtained from the five dead sheep (serial Nos. 2, 19, 30, 31 and 47), and the virus of louping-ill was recovered in low concentration from only one of the five, serial No. 47. It is probable that the virus recovered from this sheep had remained alive since immunisation.

5. *The Mortality Incidence in the Control Sheep.*

Of the 25 control sheep, 13 died—a mortality of 52 per cent. No fewer than nine of the thirteen controls which died are accounted for during the " composite " febrile phase, whereas only one of the immunised sheep died during the same period.

The other four sheep (serial Nos. 65, 67, 69 and 73) died of a braxy-like disease ; a bacteriological examination of one of these sheep (serial No. 73) was made, and *Vibrion septique* in pure culture was isolated from the heart blood and peritoneal fluid. The organism was identified morphologically, culturally and serologically.

Four sheep (serial Nos. 61, 66, 78 and 79) died soon after giving birth to dead lambs. Pregnancy was unsuspected, but the births occurred during a febrile reaction in each case, and it is probable that the febrile condition was the actual cause of the premature parturition and the subsequent death of the sheep.

Material for diagnosis was obtained from the central nervous system of five sheep (serial Nos. 60, 69, 71, 75 and 82), and the virus of louping-ill was recovered from two (serial Nos. 71 and 75). Our records show that these were the only sheep which had shown symptoms regarded by us as typical of louping-ill infection.

As a result of subsequent work, it has been found that a sheep may die of louping-ill infection without showing symptoms of involvement of the central nervous system, and in such cases the virus of louping-ill is not present in the central nervous system. The high mortality rate in the " controls " as compared with the

" immunes " would suggest that a number of cases of this type occurred during the course of the field experiment. These acute deaths, although unaccompanied by the usual clinical signs of louping-ill, we regard as really due to the infection.

6. *Loss of Weight in the Immune Sheep and Control Sheep.*

The two " composite " weight curves in Chart IV show a significant difference. A greater loss of weight occurred in the " controls " as compared with the " immunes," and this is further evidence that the " controls " were reacting to a more severe infection than were the " immunes."

Sheep Grazed on Pasture B.

Immunisation.

The death rate in the sheep during the process of immunisation necessitated the preparation of a further group of immune sheep for Pasture B. Accordingly, on March 24th, 1931, ten sheep (serial Nos. 83 to 92) were inoculated subcutaneously with 10 c.c. of 1 in 10,000 *Dried Brain* 5 in saline. On March 25th, 1931, two sheep (serial Nos. 93 and 94) were inoculated subcutaneously with 10 c.c. of 1 in 100,000 *Dried Brain* 5 in saline. On March 26th, 1931, eight sheep (serial Nos. 95 to 102) were inoculated subcutaneously with 10 c.c. of 1 in 10,000 *Dried Brain* 5 in saline.

Of these 20 sheep, four developed symptoms of louping-ill, one recovered, and three died. To the 17 survivors, seven other sheep (serial Nos. 103 to 109), already immune to louping-ill, were added (*vide* Appendix B1). The group of 24 thus formed constituted the immune sheep on Pasture B.

Immediate Results of Exposure to Natural Infection on Pasture B. (April 2nd and 8th to June 15th.)

The control group for Pasture B comprised 24 sheep, fellows to the controls on Pasture A (serial Nos. 110 to 133). The history of these sheep appears in Appendix B2. They were kept under close observation, but no temperature records were kept. The dates on which deaths occurred are shown at the bottom of Chart III. The delay in the control death rate in this experiment, as compared with Experiment A, is probably accounted for by the fact that 13 of the control sheep (serial Nos. 110, 111, 113, 114, 119, 120, 121, 124, 127, 128, 130, 131 and 133) were not transferred to the infected pasture until April 8th.

Mortality in the Immunised Sheep.

Three of the 24 immunised sheep died—a mortality of 12·5 per cent. Material for examination was obtained from two of these three sheep (serial Nos. 84 and 91). No virus was recovered from No. 84, but virus in low concentration was recovered from No. 91, and on histological examination there was evidence of slight changes in the central nervous system such as would indicate a recent

reaction to the presence of virus. For several days before it was destroyed this sheep showed symptoms suggestive of a chronic type of louping-ill. We do not hold that this was a breakdown in immunity, but regard the case as most probably a recrudescence of infection, the sheep never having overcome the virus introduced for immunisation. The very severe reactions, followed in some cases by death, which occurred during the immunisation process, support this view. In the case of the third sheep (serial No. 95) pleurisy was the immediate cause of death.

Mortality in the Control Sheep.

Thirteen of the 24 control sheep died—a mortality of 52·4 per cent. Prior to death one of these 13 sheep showed symptoms suggestive of louping-ill infection. Virus was recovered from the central nervous system and other tissues (*vide* Appendix B2, serial No. 113), and the characteristic lesions associated with louping-ill were observed on histological examination.

In six cases (serial Nos. 114, 116, 120, 121, 123 and 159) death was attributed to a braxy-like disease, and a bacteriological examination of one of these (serial No. 114) yielded *Vibrion septique* in pure culture from the stomach wall and heart blood.

Six sheep (serial Nos. 115, 118, 125, 127, 128 and 129) died from undetermined causes. None showed symptoms suggestive of involvement of the central nervous system.

The Testing of Sheep Grazed on Pastures A and B after their Return to the Laboratory (June 15th to August 16th).

Of the 97 sheep grazed on Pastures A and B there were 63 survivors ; these were returned to the laboratories on June 15th and comprised 40 " immunes " and 23 " controls."

It was decided to test these sheep for (*a*) immunity to the virus of louping-ill, and (*b*) immunity to the infective agent of " tick-borne fever."

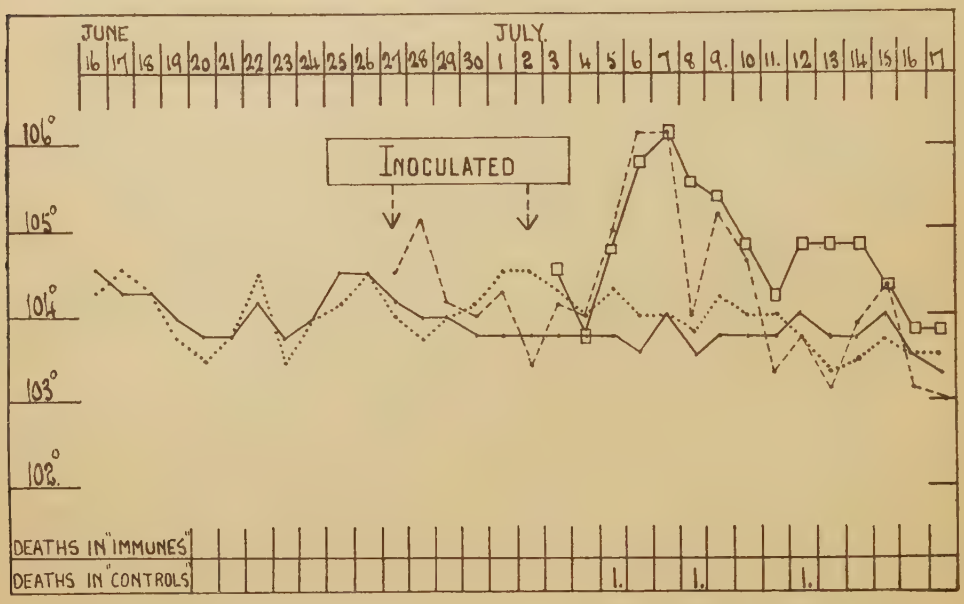
The Test for Immunity to the Virus of Louping-ill.

By inoculation of both groups of sheep with the virus of louping-ill, we hoped to prove that the " immune " sheep still possessed immunity, and to determine if any of the control sheep had developed immunity as a result of their exposure to natural infection. Accordingly, the 63 sheep, with two normal sheep, were inoculated subcutaneously on June 27th with 10 c.c. of 1 in 10,000 *Dried Brain* 5. This dose was decided upon in view of the recognised danger of using a test dose which might prove too severe. Unfortunately on this occasion the two control sheep did not react, and so on July 2nd all 65 sheep, with three other normal sheep, were injected subcutaneously with 10 c.c. of 1 in 1,000 *Dried Brain* 5. Following this inoculation the two original controls reacted, as also did the three additional controls inoculated for the first time on July 2nd.

From this result it was considered that the test dose of virus used was capable of producing a thermal response in sheep not previously immunised. A record of the result is set out in Appendix A3 and B3, and Chart V depicts the result.

CHART V.

Composite Temperature Chart of "Immunes" and "Controls" when Tested for Immunity to the Virus of Louping-ill following Exposure to Louping-ill Infection on a "Diseased Farm."



——— 40 "Immunes."
. . . . . 23 "Controls."
- - - - 2 Normals to Control the Infectivity of Virus used 27.6.31.
□——□ 3 Normals to Control the Infectivity of Virus used 2.7.31.

When tested subcutaneously with the virus of louping-ill the 40 immunised sheep *ex* Pastures A and B proved immune. Of the 23 control sheep, *ex* Pastures A and B, nine developed a temperature reaction (serial Nos. 59, 63, 76 and 80, *vide* Appendix A3 ; 110, 111, 119, 131 and 132, *vide* Appendix B3), and three of them died. The remaining 14 control sheep (serial Nos. 58, 62, 64, 68, 70, 72, 74 and 81, *vide* Appendix A3 ; 112, 117, 122, 126, 130 and 133, *vide* Appendix B3) did not react to the test dose of virus. That is, 60·9 per cent. of the surviving controls had developed an immunity. It is highly probable that these sheep became immune as a result of infection with virus acquired during the period in which they were exposed to natural infection. The evidence in support of this is contained in Appendix A1 (serial Nos. 1 to 50), which gives the history of 50 sheep of the same group which had never been grazed on infected pasture and which in every case gave a severe temperature

reaction on inoculation with the same virus. If the above recorded percentage (60·9) of the surviving controls had acquired virus while exposed to infection, then it seems highly probable that at least the same percentage of " dead " controls had been infected with virus, and that the deaths in the unprotected sheep from undetermined cause had a louping-ill infection as the primary factor causing death.

Further support for the view that sheep exposed to natural infection may acquire virus without developing typical louping-ill is found in the data set out in Tables II and III, which record the recovery of virus from ticks which fed on a control sheep (serial No. 82, Appendix A2) during the febrile phase prior to death from a braxy-like disease. This sheep developed a temperature reaction which commenced on April 9th ; it was slightly dull on April 13th, and was found dead on April 14th. Post-mortem examination revealed the presence of a necrotic lesion on the mucous membrane of the fourth stomach, which is accepted as diagnostic of " braxy." The virus of louping-ill could not be detected in the central nervous system of this sheep, and on histological examination no lesions of louping-ill could be found. It so happened that ticks replete with blood had been collected from this sheep on April 13th and 14th. In the course of feeding, the ticks had acquired blood during the febrile phase. Fifteen days later these ticks (nymphs and adults) were washed in saline, weighed and ground up in sufficient saline to make a 1 in 10 suspension. This suspension was centrifuged for 15 minutes at about 2,000 R.P.M. From the supernatant fluid progressive dilutions in saline were made to 1 in 10,000. Two mice were inoculated intracerebrally with 0·1 c.c. of each dilution, and kept under observation for 24 days. The result is set out in Table II.

TABLE II.

MICE INOCULATED WITH SALINE SUSPENSIONS OF EMULSIFIED TICKS FED ON SHEEP (SERIAL NO. 82)

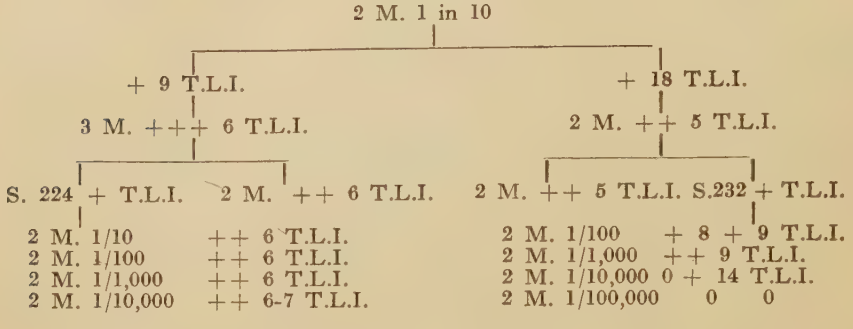
Mice inoculated ic.	*Result.*
2 M. 1 in 10	+ 9 + 18 T.L.I.
2 M. 1 in 100	0 0
2 M. 1 in 1,000	0 0
2 M. 1 in 10,000	0 0

+x = Death x days after inoculation.
0 = " No take."
M. = Mouse.
T.L.I. = Typical louping-ill.

The two mice inoculated from the 1 in 10 suspension developed symptoms typical of louping-ill infection. Virus was recovered from the central nervous system of these mice, and passed in series through mice and sheep, as indicated in Table III.

TABLE III.

RECORD OF THE INFECTIONS RESULTING FROM THE INOCULATION OF BRAIN SUSPENSION PREPARED FROM THE TWO MICE WHICH DIED FOLLOWING INOCULATION OF EMULSIFIED TICKS.



M. = Mouse.
S. = Sheep.
O. = " No take."
+x = Death x days after inoculation.
T.L.I. = Typical louping-ill.

THE TEST FOR IMMUNITY TO " TICK-BORNE FEVER."

The Nature of " Tick-borne Fever."

We have already indicated that the temperature reaction in the " immune " sheep grazed on Pasture A was caused not by the louping-ill virus, but by an infective agent or agents transmitted by ticks, and that the reaction in the control sheep so grazed might be due to the presence of the true louping-ill virus in the blood stream, or to the agent responsible for the reaction in the " immunes," or to both. Also, as already described, there was some evidence suggesting that the reactions in the two groups of sheep were not identical.

For purposes of description, we have named the reaction which occurred in the " immune " sheep " reaction of immunes," and that which occurred in the " control " sheep " reaction of controls."

Blood drawn from an " immune " sheep (serial No. 56) on April 15th, when its temperature was 107·8° F., and from a " control" sheep (serial No. 79) on April 15th, when its temperature was 107·4° F., was shown to contain no louping-ill virus, by intracerebral inoculation of sheep. By the subcutaneous inoculation of sheep with these specimens of blood we have subsequently passed the causal agent of both reactions through many generations—26 in the case of the " immune," and 27 in the case of the " control." In each series there has been produced a disease characterised by a low mortality, with an incubation period of about four days, followed by a sharp rise in temperature and a period of fever, which is often irregular and very prolonged but usually lasts about nine days.

We have now shown by transmission experiments that this reaction is a " tick-borne fever." Ticks collected from the pasture,

and ticks collected from reacting sheep, have produced a temperature reaction when allowed to feed on normal sheep. The reaction can be produced at will with ticks known to be infected, and it has been proved to be similar in its manifestation to that which occurred in the " immune " sheep while these were grazing on tick-infested ground.

" Tick-borne fever " can be readily distinguished from louping-ill by the following facts :—

(*a*) The incubation period of " tick-borne fever " is longer than that of louping-ill.

(*b*) The two diseases are immunologically distinct.

(*c*) Louping-ill mortality is high, " tick-borne fever " mortality is low.

(*d*) Mice can be infected with louping-ill but not with " tick-borne fever."

(*e*) In louping-ill infection there is an encephalomyelitis. In " tick-borne fever " infection encephalomyelitis is not present.

We believe " tick-borne fever " to be due to a specific infective agent transmitted by the tick, but the nature of the infective agent has not yet been determined. Filtration experiments have, so far, yielded negative results, and examinations for the presence of bacteria and protozoon parasites have also been attended with negative results. The type of the disease, coupled with the evidence that it is transmitted by ticks, suggests the possibility that the infective agent may be Rickettsia. This aspect of the problem is the subject of further work, and will be dealt with in future publications.

Results of Testing for Immunity to " Tick-borne Fever."

Sixty sheep (40 " immunes " and 20 " controls "), returned to the laboratory from Pastures A and B, were tested for immunity to " tick-borne fever." Blood from immune and control sheep, referred to above, and proved to contain the infective agent of " tick-borne fever " in each case, was used as follows :—

Nineteen " immunes " *ex* Pasture A inoculated with 5 c.c. of " control reactor's " blood.

Twenty-one " immunes " *ex* Pasture B inoculated with 5 c.c. of " immune reactor's " blood.

Ten " controls " *ex* Pasture B inoculated with 5 c.c. of " control reactor's " blood.

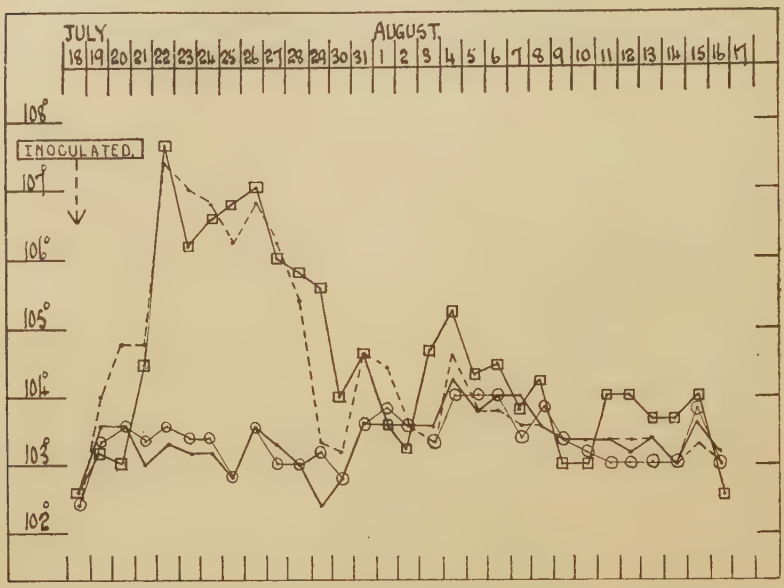
Ten " controls " *ex* Pasture A inoculated with 5 c.c. of " immune reactor's " blood.

We thus tested 21 louping-ill immune sheep with " immune reactor's " blood and 19 with " control reactor's " blood, also ten control sheep with " immune reactor's " blood, and ten control sheep with " control reactor's " blood.

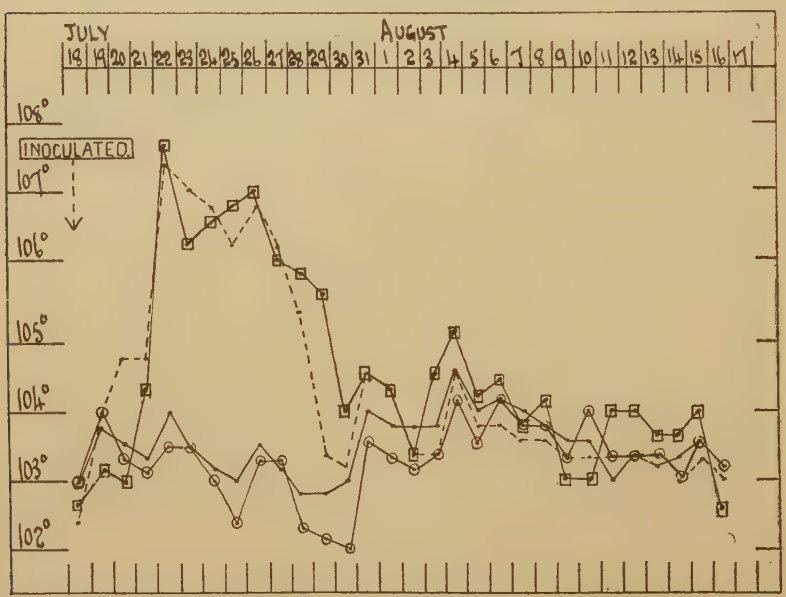
The result of these tests is shown in Appendices A3 and B3. The reactions which occurred in the eight sheep used to control the infectivity of the blood are described in Appendices C2 and C3, and the results are depicted in Charts VI and VII.

CHARTS VI AND VII.

COMPOSITE TEMPERATURE CHART OF "IMMUNES" AND "CONTROLS" WHEN TESTED FOR IMMUNITY TO TICK BORNE FEVER FOLLOWING EXPOSURE TO LOUPING ILL INFECTION ON A "DISEASED FARM."



——— 19 "Immunes" Ex Pasture A. Tested with "C.R." Blood.
- - - - 4 Normal Sheep to Control the Infectivity of the "C.R." Blood.
○——○ 21 "Immunes" ex Pasture B. Tested with "I.R." Blood.
□——□ 4 Normal Sheep to Control the Infectivity of the "I.R." Blood.



——— 10 "Controls" ex Pasture B. Tested with "C.R." Blood.
- - - - 4 Normal Sheep to Control the Infectivity of the "C.R." Blood.
○——○ 10 "Controls" ex Pasture A Tested with "I.R." Blood.
□——□ 4 Normal Sheep to Control the Infectivity of the "I.R." Blood.

For abbreviations see p. 140.

All the sheep, with the exception of one (serial No. 110), after return from the field experiment were immune when tested with these two types of infective blood, whereas sheep which had not been on the experimental farm gave a marked thermal response. In other words, the temperature reaction which occurred in both the immune and control sheep while on the experimental pasture, and which has been subsequently proved to be a " tick-borne fever," was followed by an immunity to the inoculation of blood known to be infective. The only death which occurred (serial No. 110) was inexplicable.

Discussion.

A field experiment is described in which a group of 48 sheep rendered immune to the virus of louping-ill, together with a group of 49 susceptible sheep (controls), were grazed on a pasture where louping-ill was known to occur. During the period of exposure to infection the temperatures of 24 sheep in each group were recorded daily ; also 15 of the " immune " and 15 " controls " were weighed twice weekly. All the sheep became infested with ticks, and in every sheep (immune and control) of which a temperature record was kept a febrile reaction developed.

It has been shown that the reaction in the " immune " sheep is attributable to an infective agent transmitted by the ticks, and the condition has been provisionally named " tick-borne fever."

The cause of the reaction in the " control " sheep was attributable, in at least a large proportion of cases, to the infective agent of " tick-borne fever," together with the virus of louping-ill ; this conclusion is supported by the following facts :—

(*a*) A greater loss of weight occurred in the " controls " as compared with that in the " immunes."

(*b*) The febrile affection in the "controls " had a shorter period of incubation than that in the " immunes."

(*c*) 60·9 per cent. of the surviving " controls " had acquired immunity to the virus of louping-ill, and all had acquired immunity to " tick-borne fever."

(*d*) The presence of virus in the blood of a sheep prior to death from a braxy-like disease has been demonstrated. This sheep showed no symptoms of louping-ill and virus could not be demonstrated in the central nervous system, and no lesions of louping-ill were present.

The following is the analysis of the mortality incidence in the immunised and control sheep.

14	Healthy immune sheep :	mortality, nil.	0 per cent.
34	Convalescent immune sheep :	mortality, 8	23·5 „
Total 48	Immunised sheep :	mortality, 8	16·6 „
49	Control sheep :	mortality, 26	53·0 „

The mortality incidence in the 48 immunised sheep is accounted for by the fact that many of them were in a debilitated convalescent state following immunisation with living virus, and that death occurred

in eight of them was not unexpected. The remaining 14 immunised sheep had completely recovered after immunisation and, although each developed a febrile reaction indicative of " tick-borne fever " infection, none of these sheep died.

We did not examine the tissues of all control sheep which died because many showed no clinical evidence of central nervous system infection which we believed at that time to be the *only* characteristic of louping-ill. We now know that the virus of louping-ill may cause the death of sheep which present no symptoms of central nervous system infection. Accordingly, the fact that many of the surviving control sheep had acquired immunity to louping-ill indicates that a natural infection with the virus had occurred during the period of exposure on the infected pasture, and it is probable that many of the control sheep which died were primarily infected with the virus of louping-ill and succumbed to this infection.

These findings suggest that many sheep on a louping-ill farm acquire virus during the louping-ill season. Some develop a mild reaction, recover, and are subsequently immune. Some develop symptoms indicative of infection of the central nervous system, and it has been our experience that in these cases lesions of louping-ill can be demonstrated in, and virus recovered from, the brain and spinal cord. Our experience, however, also indicates that in many other cases no symptoms such as have been previously associated with louping-ill develop, and the sheep are found dead from undetermined cause.

On many louping-ill farms " tick-borne fever " probably occurs in individual sheep concurrently with louping-ill infection, and this fact may account for some of the difficulties experienced by previous investigators in establishing the etiology of louping-ill. From the point of view of immunisation against louping-ill, it is fortunate that the mortality incidence following " tick-borne fever " infection *per se* is low. The significance, if any, of the association of the two diseases is not clear, and is the subject of further study. As a result of subsequent work, it has been established that the majority of the ticks collected from this experimental pasture harboured the infective agent of " tick-borne fever " while only a few have been proved to harbour the virus of louping-ill.

In consideration of the heavy mortality that occurred in the control sheep, and of the fact that a number of the survivors had acquired immunity to louping-ill, it is possible that the process of " acclimatisation " of the sheep on many of the hill farms in Scotland represents in reality the development of immunity to the virus of louping-ill, but during the process of " acclimatisation " a heavy annual toll on the sheep population of " diseased " farms is exacted. If this immunity can be established by reliable artificial methods, it is probable that louping-ill will be brought under effective control. The part which " tick-borne fever " infection may play in " acclimatisation " has yet to be determined.

Conclusions.

1. It may now be taken as proved that louping-ill is due to a distinct filter passing virus.

2. The clinical manifestations of typical louping-ill are due to infection of the central nervous system with this virus.

3. The virus has been detected in the blood in cases in which symptoms of louping-ill did not subsequently develop. The symptoms of infection in such cases is a febrile reaction occasionally followed by death.

4. The above data suggest that for the occurrence of typical louping-ill invasion of the central nervous system by the virus is essential. It would appear that accessory conditions favour such invasion, *e.g.* the occurrence of tick-borne fever. (See No. 7.)

5. Recovery from the infection, either naturally or experimentally produced, results in immunity to louping-ill.

6. The evidence so far indicates that the use of a living virus for prophylactic immunisation is not a safe procedure.

7. As a result of the experimental investigation of louping-ill it has been shown that ticks (*Ixodes ricinus*) which transmit the virus also harbour another infective agent which causes febrile disease in sheep. Cross immunity experiments have shown that this disease is distinct from louping-ill. The nature of the causal agent is so far unknown.

The Appendix.

The abbreviations used in this appendix and elsewhere in this report are shown on page 140.

There are three appendices: A, B and C. Appendices A and B follow the same sequence as the text of the paper.

Appendix A deals with all sheep grazed on Pasture A and is divided into three phases.

Each sheep has been given a serial number and by following the serial number of any surviving sheep its history can be traced through each phase.

Appendix B is compiled on the same lines as " Appendix A," and deals with the sheep grazed on Pasture B.

Appendix C.

I. The history of five normal sheep acting as controls to prove the infectivity of virus used for testing the " Immune " and " Control " sheep grazed on Pastures A and B for immunity to " louping-ill."

II. The history of four normal sheep acting as controls to prove the infectivity of the " Control reactors " blood, which was used for testing the " Immune " sheep grazed on Pasture A and the " Control " sheep grazed on Pasture B.

III. The history of four normal sheep acting as controls to prove the infectivity of the " Immune reactors " blood, which was used for testing the " Immune " sheep grazed on Pasture B and the " Control " sheep grazed on Pasture A.

APPENDIX A I.

The History of the Sheep during Immunisation for Pasture A.

Ser. No.	*S. No.*	*Result of subcutaneous inoculation with 10 c.c. D.B. 5. Date 16/3/31.*	
1	80	T.R., T.L.I.	+ 10
2	81	T.R., V.D.	L
3	82	T.R.	+ 12
4	83	T.R., T.L.I., 9th, 10th day.	+ 11
5	84	T.R., T.L.I., 9th, 10th day.	+ 11
6	85	T.R.	L
7	86	T.R.	L
8	87	T.R., T.L.I., 9th day.	+ 10
9	88	T.R., V.D.	L
10	89	T.R., T.L.I., 12th day.	+ 12
11	90	T.R., T.L.I., 9th day.	+ 10
12	91	T.R., D.	L
13	92	T.R., D.	L
14	93	T.R., V.D., 16th to 21st day.	+ 22
15	94	T.R., T.L.I., 11th day.	+ 12
16	95	T.R.	L
17	96	T.R., T.L.I., 8th, 9th day.	+ 10
18	97	T.R., T.L.I., 10th day.	+ 11
19	98	T.R., V.D.	L
20	99	T.R., T.L.I., 12th day.	+ 12
21	100	T.R., T.L.I., 11th day.	+ 11
22	101	T.R., T.L.I., 10th day.	+ 11
23	102	T.R., T.L.I., 8th day.	+ 9
24	103	T.R., T.L.I., 8th day.	+ 8
25	104	T.R., V.D.	+ 17
26	105	T.R., D.	L
27	106	T.R., D.	L
28	107	T.R., T.L.I., 10th day.	+ 11
29	108	T.R., D.	L
30	109	S.T.R., D.	L
31	110	T.R., V.D.	L
31	111	T.R., T.L.I., 10th day.	+ 11
33	112	T.R., V.D.	+ 28
34	113	T.R.	L
35	114	T.R., T.L.I., 10th day.	+ 11
36	115	T.R., T.L.I., 5th, 6th day.	+ 7
37	116	T.R., T.L.I., 10th day.	+ 10
38	117	T.R., T.L.I., 9th day.	+ 10
39	118	T.R., T.L.I., 8th day.	+ 9
40	119	T.R., T.L.I., 12th day.	+ 12
41	120	T.R., T.L.I., 5th, 6th day.	+ 7
42	121	T.R., T.L.I., 6th day.	+ 6
43	122	T.R., T.L.I., 9th day.	+ 50
44	123	T.R., T.L.I., 10th, 11th day.	+ 12
45	124	T.R.	+ 11
46	125	T.R., T.L.I., 9th day.	+ 9
47	126	T.R., V.D.	L
48	127	T.R., D.	L
49	128	T.R.	L
50	129	T.R., T.L.I., 12th, 13th day.	+ 14

Ser. No.	*S. No.*	*Date.*	*Inoculum*	*Result.*	
51	20	Feb. 11	10 c.c. 1/1,000,000 D.B.5. sc.	N.T.R.	
		Mar. 4	1 c.c. 1/100 D.B.5. ic.	T.R.	L
52	21	Feb. 11	10 c.c. 1/1,000 D.B. 5. sc.	T.R.	
		Mar. 4	1 c.c. 1/100 D.B.5. ic.	N.T.R.	L
53	29	Feb. 11	10 c.c. 1/100 D.B.5. sc.	T.R., D.	
		Mar. 4	1 c.c. 1/100 D.B.5. ic.	N.T.R.	L
54	986	Feb. 11	10 c.c. 1/10,000 D.B.5. sc.	T.R., D.	
		Mar. 4	1 c.c. 1/100 D.B.5. ic.	N.T.R.	L
55	989	Feb. 11	10 c.c. 1/100,000 D.B.5. sc.	T.R.	
		Mar. 4	1 c.c. 1/100 D.B.5. ic.	N.T.R., D.	L
56	991	Feb. 11	10 c.c. 1/100,000 D.B.5. sc.	T.R.	
		Mar. 4	1 c.c. 1/100 D.B.5. ic.	N.T.R.	L
57	994	Feb. 11	10 c.c. 1/10 D.B.5. sc.	T.R., S.D.	
		Mar. 4	1 c.c. 1/100 D.B.5. ic.	N.T.R.	L

APPENDIX A II.

THE HISTORY OF THE "IMMUNES" WHILE EXPOSED TO INFECTION ON PASTURE A. PERIOD APRIL 2nd TO JUNE 15th.

	Ser. No.	S. No.	Date T. rose.	Date and height of first peak.	T. in ° F. fluctuated between.	T. normal.	Symptoms. Post-mortem. Material examined.	Result.
Convalescent Immune Sheep.	2	81	Ap. 10	13th 105·4	101·8-105·8	—	Ap. 17-29 weak and dull. Ap. 30 died. Cerebrum, cerebellum. cervical, dorsal and lumbar cords for diagnosis. No virus recovered. Slight — infiltration into meninges over cerebellum.	+28
	6	85	Ap. 11	13th 107	103-106·4	May 22	—	L
	7	86	Ap. 8	9th 107·6	102·6-107	May 13	—	L
	9	88	Ap. 10	11th 106·4	102-107·4	May 28	—	L
	12	91	Ap. 10	12th 107·2	103-106	May 7	—	L
	13	92	Ap. 9	10th 106·4	102-107	May 27	—	L
	16	95	Ap. 9	14th 107·2	102-107·2	May 16	—	L
	19	98	Ap. 11	12th 107·2	103-106·8	Ap. 27	May 22-26 very weak and dull. May 27 dead. Cerebrum, cervical and lumbar cord for diagnosis. No virus recovered. No lesions found.	+55
	26	105	Ap. 8	11th 107	102-107·2	May 16	—	L
	27	106	Ap. 14	16th 107·6	102·4-106	May 2	—	L
	29	108	Ap. 10	13th 107·2	103-107·2	May 5	—	L
	30	109	Ap. 13	15th 106·6	102·8-107·2	Ap. 20	May 6 T. fell to 101° F. May 6-7 dull and weak. May 8 dead. Cerebrum, cerebellum, cervical, dorsal and lumbar cords for diagnosis. No virus recovered.	+36
	31	110	Ap. 8	12th 107	104-106	—	T. remained high till Ap. 20. Fell gradually to 95·6 on Ap. 27. Ap. 20-26 weak. Ap. 27 dead. Cerebrum, cerebelum, cervical dorsal and lumbar cords for diagnosis. No virus recovered.	+25
	34	113	Ap. 10	13th 106	102·6-106·4	May 4	—	L
	47	126	Ap. 12	13th 106	103·2-105·6	Ap. 18	Ap. 18-May 1 dull and weak. May 2 found dead. Cerebrum, cerebellum, cervical, dorsal and lumbar cords for diagnosis. Virus recovered from 1 out of 6 mice inoculated.	+30
Healthy Immune Sheep.	48	127	Ap. 10	11th 107	103-107·8	June 1	—	L
	49	128	Ap. 19	21st 107·8	102-106	May 18	—	L
	51	20	Ap. 11	13th 107·4	104-107·6	June 4	—	L
	52	21	Ap. 11	14th 107·6	104-107·8	May 8	—	L
	53	29	Ap. 8	10th 108	103-107·6	May 23	—	L
	54	986	Ap. 11	12th 107·4	104-108	Ap. 28	—	L
	55	989	Ap. 11	12th 107·6	102-107	May 19	—	L
	56	991	Ap. 9	13th 108	103·2-108·2	May 14	—	L
	57	994	Ap. 7	10th 106·8	102-107·6	May 4	—	L

APPENDIX A II (*Continued*).

THE HISTORY OF THE "CONTROLS" WHILE EXPOSED TO INFECTION ON PASTURE A. PERIOD APRIL 2nd TO JUNE 15th.

Ser. No.	*S. No.*	*Date T. rose.*	*Date and height of first peak.*	*T. in ° F. fluctuated between.*	*T. normal.*	*Symptoms. Post-mortem. Material examined.*	*Result.*
58	130	Ap. 8	12th 107·4	102-107·6	May 14	Ap. 21-25 dull and trembling.	L
59	132	Ap. 9	11th 106·4	104-107·4	May 4	Ap. 12 slightly dull.	L
60	133	Ap. 9	11th 106·6	101·4-107·4	Ap. 30	Ap. 10-May 28 S.D. and weak. May 28 T. fell to 99° F. Found dead same day. Cerebrum, cervical and lumbar cords for diagnosis. No virus recovered. No lesions found.	+56
61	134	Ap. 7	9th 106·8	103-106	Ap. 18	Ap. 13 dead lamb born. Ap. 14-22 dull and weak. Ap. 23 found dead.	+21
62	135	Ap. 6	9th 107	102·6-107	May 10	—	L
63	138	Ap. 4	8th 107·2	102-107·8	May 18	—	L
64	139	Ap. 10	11th 107·4	101·6-107·4	May 16	—	L
65	142	Ap. 7	8th 106·8	105·6-106·8	Ap. 20	2nd rise in T. Ap. May 1 105·6° F., May 2 105·4° F., May 3 106° F., May 4 found dead. Appearance of anærobic infection of abdominal cavity. Braxy type of case.	+32
66	143	Ap. 7	8th 107	104·8-106·8	—	T. remained high till Ap. 16 when dead lamb was born. Ap. 17 T. fell to 101·4° F. Found dead same day.	+15
67	145	Ap. 15	17th 106·6	102·6-108	—	T. fell from 106° F. on Ap. 25 to 98·4° F. on Ap. 26 when sheep was lying on its side unable to rise. Ap. 27 found dead. Acute enteritis.	+25
68	149	Ap. 7	9th 107	104·4-107	Ap. 18	—	L
69	150	Ap. 7	9th 106·8	—	—	Ap. 10 105·6° F. found dead. Braxy type of case. Cerebrum, cerebellum, cervical, dorsal and lumbar cords for diagnosis. No virus recovered. No lesions found.	+ 9
70	152	Ap. 16	17th 107·6	100·8-106·6	May 15	May 10—June 15 very weak.	L

Ser. No.	*S. No.*	*Date T. rose.*	*Date and height of first peak.*	*T. in ° F. fluctuated between.*	*T. normal* n	*Symptoms. Post-mortem. Material examined.*	*Result.*
71	153	Ap. 7	8th 107·2	103-106·4	—	May 12, 13, 14 very dull, straddling and irregular gait. Found stretched out showing occasional convulsions. May 14 destroyed. Cerebrum, cerebellum, cervical, dorsal and lumbar cords for diagnosis. Virus recovered highly infective dilution 1 in 10,000. Lesions of L.I. in C.N.S.	+42
72	158	Ap. 8	13th 107·6	102·6-107	May 26	—	L
73	160	Ap. 8	10th 106·8	105·2-107	—	T. fell gradually to 102·4° F. on Ap. 22. Dull and weak. Found dead. Braxy-like disease. *Vibrion septique* recovered from H.B. and peritoneal fluid. Identified morphologically, culturally and serologically.	+20
74	164	Ap. 8	11th 106·8	104·4-107	May 24	—	L
75	167	Ap. 8	10th 107	105-107·4	—	T. high till Ap. 21 sudden fall to 100. 6° F. on Ap. 23. Lying on its side unable to rise. Padling movements of limbs. Slight salivation. Tremors of lips. Destroyed. Virus recovered from cerebrum, cerebellum, hippocampus, spinal ganglia, cervical, dorsal and lumbar cords, sciatic nerve, pancreas and adrenal gland. Highest infective dilution, cerebellum 1 in 100,000. Lesions of L.I. throughout the C.N.S.	+21
76	168	Ap. 11	12th 107	102-107·4	May 24	—	L
77	169	Ap. 7	10th 107·4	103·8-106·8	May 4	2nd T.R. from May 13-23 100·8, 102·2 105·8, 106·6, 103·8, 102·4, 102·2, 102·2, 101·6, 100·4, 100. During this period very dull and weak. May 23 found dead. Carcase œdematous.	+51

Ser. No.	*S. No.*	*Date T. rose.*	*Date and height of first peak.*	*T. in ° F. fluctuated between.*	*T. normal.*	*Symptoms. Post-mortem. Material examined.*	*Result.*
78	171	Ap. 8	10th 106·8	104·8-105·8	—	Dead lamb born Ap. 16, found dead same day.	+14
79	173	Ap. 11	12th 106·4	—	—	T. reached its height Ap. 15 107·4, 16 107, 17 104·6, 18 98 when sheep lambed. Ap. 19 found dead.	+17
80	175	Ap. 8	9th 106·8	102·2-107	June 4	—	L
81	177	Ap. 7	12th 107·6	102·4-107·4	May 24	Ap. 12-19 dull.	L
82	178	Ap. 9	10th 107	—	—	Ap. 11 T. 107, 12 106, 13 104·6 when sheep was slightly dull. Ap. 14 found dead. Braxy type of case. Necrotic area in the fourth stomach. Cerebrum, cerebellum cervical, dorsal and lumbar cords for diagnosis. No virus recovered. No lesions found.	+12

APPENDIX A III.

PERIOD JUNE 16th TO AUGUST 16th.

The history of the " immunes " *ex* Pasture A when tested for :—

1.—Immunity to " louping-ill."
2.—Immunity to " tick-borne fever."

Ser. No.	*S. No.*	1.—" *Louping-ill* " *Immunity Test.* *Result of two inoculations with D.B. 5. sc.—one on 27/6/31, the other on 2/7/31.*	2.—" *Tick-borne fever* " *Immunity Test.* *Result of sc. inoculation with 5 c.c. C.R. blood on 18/7/31.*
6	85	N.T.R.	N.T.R.
7	86	N.T.R.	N.T.R.
9	88	N.T.R.	N.T.R.
12	91	N.T.R.	N.T.R.
13	92	N.T.R.	N.T.R.
16	95	N.T.R.	N.T.R.
26	105	N.T.R.	N.T.R.
27	106	N.T.R.	N.T.R.
29	108	N.T.R.	N.T.R.
34	113	N.T.R.	N.T.R.
48	127	N.T.R.	N.T.R.
49	128	N.T.R.	N.T.R.
51	20	N.T.R.	N.T.R.
52	21	N.T.R.	N.T.R.
53	29	N.T.R.	N.T.R.
54	986	N.T.R.	N.T.R.
55	989	N.T.R.	N.T.R.
56	991	N.T.R.	N.T.R.
57	994	N.T.R.	N.T.R.

PERIOD JUNE 16th TO AUGUST 16th.

The history of the " controls " *ex* Pasture A when tested for :—

1.—Immunity to " louping-ill."

2.—Immunity to " tick-borne fever."

Ser. No.	*S. No.*	1.—" *Louping-ill* " *Immunity Test.* *Result of two inoculations with D.B. 5 sc.—one on 27/6/31, the other on 2/7/31.*	2.—" *Tick-bourne fever.*" *Immunity Test.* *Result of sc. inoculation with 5 c.c. I.R. blood on 18/7/31.*
58	130	N.T.R.	N.T.R.
59	132	T.R. July 8 died. No virus recovered.	
62	135	N.T.R.	N.T.R.
63	138	T.R.	N.T.R.
64	139	N.T.R.	N.T.R.
68	149	N.T.R.	N.T.R.
70	152	N.T.R. Very weak and dull.	N.T.R. June 29 sheep in debilitated condition. Destroyed.
72	158	N.T.R.	N.T.R.
74	164	N.T.R.	N.T.R.
76	168	T.R. Symptoms of T.L.I. July 11. July 13 destroyed. No virus recovered from C.N.S. Lesions of L.I. in cervical and lumbar cords.	
80	175	T.R. June 29-July 9. July 4 sheep very dull.	N.T.R.
81	177	N.T.R.	N.T.R.

APPENDIX B I.

The History of the Sheep during Immunisation for Pasture B.

Ser. No.	*S. No.*	*Date of Injection.*	*Inoculum*	*Result.*	
83	68	24/3/31	10 c.c. 1/10,000 D.B. 5. sc.	N.T.R.	L
84	62	„	„	N.T.R.	L
85	63	„	„	N.T.R.	L
86	66	„	„	T.R.	L
87	72	„	„	T.R.	L
88	73	„	„	T.R., T.L.I. 8th–9th day	L
89	74	„	„	T.R.	L
90	76	„	„	T.R.	L
91	78	„	„	T.R.	L
92	69	„	„	T.R., T.L.I. 9th day	+10
93	65	25/3/31	10 c.c. 1/100,000 D.B. 5. sc.	N.T.R.	L
94	70	„	„	N.T.R.	L
95	997	26/3/31	10 c.c.1/10,000 D.B. 5. sc.	T.R., S.D.	L
96	990	„	„	T.R., D.	L
97	75	„	„	T.R.	L
98	988	„	„	T.R., D.	L
99	983	„	„	T.R.	L
100	981	„	„	T.R., V.D.	L
101	64	„	„	T.R., T.L.I. 7th day	+ 8
102	993	„	„	T.R., T.L.I. 10-12th day	+13
103	71	11/3/31	Suspension of mouse brain strain ewe 5 1929 1 c.c.ic.	T.R., V.D. Slight tremors of head March 18.	L
104	996	11/2/31	10 c.c. 1/1,000 D.B. 5 sc.	T.R.	
		4/3/31	1 c.c. 1/100 D.B. 5. ic.	N.T.R.	L
105	42	11/2/31	10 c.c. 1/100 D.B. 5. sc.	V.S.T.R.	
		4/3/31	1 c.c. 1/100 D.B. 5. ic.	N.T.R.	L
106	987	11/2/31	10 c.c. 1/10,000 D.B. 5. sc.	V.S.T.R.	
		4/3/31	1 c.c. 1/100 D.B. 5. ic.	S.D.	L
107	*36	8/1/31	20 c.c. L.I. Vaccine sc.	N.T.R.	
		22/1/31	20 c.c. L.I. Vaccine sc.	N.T.R.	
		5/2/31	1 c.c. 1/100 D.B. 5. ic.	N.T.R.	
		4/3/31	15 c.c. 1/10 D.B. 12. I.M.	N.T.R.	L
108	37	8/1/31	10 c.c. 1/100 D.B. 5. sc.	T.R., T.L.I. 11-14 day.	
		5/2/31	1 c.c. 1/100 D.B. 5. ic.	N.T.R.	
		4/3/31	15 c.c. 1/10 D.B. 12. I.M.	N.T.R.	L
109	44	22/1/31	10 c.c. 1/100 D.B. 5. sc.	T.R., S.D.	
		5/2/31	1 c.c. 1/100 D.B. 5. ic.	N.T.R.	
		4/3/31	15 c.c. 1/10 D.B. 12. I.M.	N.T.R.	L

* The vaccine used for immunising this sheep was an experimental product, the nature of which will be described in a later publication.

APPENDIX B II.

THE HISTORY OF THE "IMMUNES" WHILE EXPOSED TO INFECTION ON PASTURE B.

PERIOD APRIL 2nd TO JUNE 15th.

	Ser. No.	*S. No.*	*Symptoms.*	*Post-mortem.*	*Material examined.*	*Result.*
Convalescent Immune Sheep	83	68		—		L
	84	62	May 13-27 dull, chronic diarrhœa. May 27 found dead.		Cerebrum, cervical, dorsal and lumbar cords for diagnosis No virus recovered. No lesions found.	+55
	85	63		—		L
	86	*66		—		L
	87	*72		—		L
	88	*73		—		L
	89	*74		—		L
	90	*76		—		L
	91	*78	May 2 lame. May 3 unable to rise. May 5-7 paddling movements of limbs. May 8 destroyed.		Virus recovered from 1 of 2 mice inoculated. Mild lesions of louping-ill in C.N.S.	+30
	93	65		—		L
	94	70		—		L
	95	*997	May 2 very lame. Disinclined to move. May 5 found dead.		P.M. septic pleurisy.	+27
	96	*990		—		L
	97	*75	May 19-20 sheep dull. June 9-10 very dull.			L
	98	*988		—		L
	99	*983		—		L
	100	*981		—		L
Healthy Immune Sheep	103	71		—		L
	104	996		—		L
	105	42		—		L
	106	987		—		L
	107	*36		—		L
	108	*37		—		L
	109	*44		—		L

THE HISTORY OF THE "CONTROLS" WHILE EXPOSED TO INFECTION ON PASTURE B.

PERIOD APRIL 2nd TO JUNE 15th.

Ser. No.	*S No.*	*Symptoms. Post-mortem. Material examined.*	*Result.*
110	*131	Ap. 28-May 13 slightly dull. Chronic diarrhœa.	L
111	*136	—	L
112	137	—	L
113	*140	May 9 lying alone. Inco-ordination of gait. Occasional periods of stupor. May 11 found dead. On the ground there was evidence that paddling movements of the limbs had occurred. Virus recovered from cerebrum, cerebellum, hippocampus, spinal ganglia, cervical, dorsal and lumbar cords, sciatic and median nerves, pancreas and adrenal gland. Highest infective dilution cerebellum 1 in 100. Lesions of louping-ill throughout C.N.S.	+33
114	*141	May 7 found dead. P.M. appearance suggestive of anærobic infection of abdominal cavity. Braxy type of case. *Vibrion septique* recovered from H.B. and stomach wall. Identified morphologically, culturally and serologically.	+29
115	144	May 9 dull. May 10 found dead. No definite cause of death.	+38
116	146	Ap. 20 just able to stand. Found dead same day. P.M. appearance suggestive of anærobic infection of abdominal cavity. Braxy type of case.	+18
117	147	—	L
118	148	Ap. 29-May 2 weak. May 2 found dead. P.M. organs anæmic.	+30
119	*151	—	L
120	*154	Ap. 20 found dead. P.M. appearance suggestive of anærobic infection of abdominal cavity. Braxy type of case.	+12
121	*155	Ap. 29 diarrhœa. May 2 found dead. P.M. appearance suggestive of anærobic infection of abdominal cavity. Braxy type of case.	+24
122	156	—	L
123	157	Ap. 24 found dead. P.M. suggestive of anærobic infection of abdominal cavity. Braxy type of case.	+22
124	*159	Ap. 18 found dead. P.M. appearance suggestive of anærobic infection of abdominal cavity. Braxy type of case.	+10
125	161	Ap. 20 found dead. P.M. organs anæmic. Slight parasitic infestation of lungs.	+18
126	162	—	L
127	*163	Ap. 29-May 25 weak. May 25 found dead. P.M. organs anæmic.	+47
128	*165	Ap. 20 found dead. P.M. organs anæmic.	+12
129	166	May 21-23 dull. May 23 found dead. P.M. organs anæmic. Excess of fluid in serous cavities.	+51
130	*170	—	L
131	*172	—	L
132	174	May 2 dull.	L
133	*176	—	L

* Sheep which were not put on to the pasture until Ap. 8. The others were put on the pasture on Ap. 2.

APPENDIX B III.

PERIOD JUNE 16th TO AUGUST 16th.

The history of the " immunes " *ex* Pasture B when tested for :—

1.—Immunity to " louping-ill."

2.—Immunity to " tick-borne fever."

Ser. No.	*S. No.*	1.—" *Louping-ill* " *Immunity Test.* *Result of two inoculations with D.B.5. sc.—one on 27/6/31, the other on 2/7/31.*	2.—" *Tick-borne fever* " *Immunity Test.* *Result of sc. inoclation with 5 c.c. I.R. blood on 18/7/31.*
83	68	N.T.R.	N.T.R.
85	63	T.R. Not considered to be due to the virus. Quite atypical, occurred 14 days after inoculation.	N.T.R.
86	66	N.T.R.	N.T.R.
87	72	N.T.R.	N.T.R.
88	73	N.T.R.	N.T.R.
89	74	N.T.R.	N.T.R.
90	76	N.T.R.	N.T.R.
93	65	N.T.R.	N.T.R.
94	70	N.T.R.	N.T.R.
96	990	N.T.R.	N.T.R.
97	75	N.T.R.	N.T.R.
98	988	N.T.R.	N.T.R.
99	983	N.T.R.	N.T.R.
100	981	N.T.R.	N.T.R.
103	71	N.T.R.	N.T.R.
104	996	N.T.R.	N.T.R.
105	42	N.T.R.	N.T.R.
106	987	N.T.R.	N.T.R.
107	36	N.T.R.	N.T.R.
108	37	N.T.R.	N.T.R.
109	44	N.T.R.	N.T.R.

PERIOD JUNE 16th TO AUGUST 16th.

The history of the " controls " *ex* Pasture B when tested for :—

1.—Immunity to " louping-ill."

2.—Immunity to " tick-borne fever."

Ser. No.	*S. No.*	1.—" *Louping-ill* " *Immunity Test.* *Result of two inoculations with D.B. 5. sc.—one on 27/6/31, the other on 2/7/31.*	2.—" *Tick-borne fever* " *Immunity Test.* *Result of sc. inoculation with 5 c.c. C.R. blood on 18/7/31.*
110	131	T.R. commenced June 29. Fluctuated between 103-106·6. July 12 normal.	July 21 T.R. commenced. July 22 106·4, 23 105, when sheep died. No virus recovered. No lesions found.
111	136	July 1 T.R. commenced. 2 107·4, 3 106·4, 4 103·8, very dull, head handing. July 5 found dead. No virus recovered.	
112	137	N.T.R.	N.T.R.
117	147	N.T.R.	N.T.R.
119	151	June 29 T.R. commenced. July 2 106·2. Fluctuated between 103·4-105·8 till July 14	N.T.R.
122	156	N.T.R.	N.T.R.
126	162	N.T.R.	N.T.R.
130	170	N.T.R.	N.T.R.
131	172	July 4 T.R. commenced July 7 105·8. Fluctuated between 104·2-105·8 till July 14.	N.T.R.
132	174	T. rose to 106·4 on July 5.	N.T.R.
133	176	N.T.R.	N.T.R.

APPENDIX C I.

THE HISTORY OF FIVE NORMAL SHEEP TO CONTROL THE INFECTIVITY OF THE VIRUS OF LOUPING-ILL USED WHEN TESTING THE IMMUNITY OF THE "IMMUNES" AND "CONTROLS" *ex* PASTURES A AND B.

Ser. No.	*S. No.*	*Result of two inoculations with D.B. 5. sc., one on 27/6/31, the other on 2/7/31.*
134	230	T.R. commenced July 5. July 6 106·4. Fluctuated between 104-107·2 till July 13 when it returned to normal.
135	223	T.R. commenced July 4. July 5 106. July 6 106. July 7 106·4. July 8 normal.
136	219	T.R. commenced July 4. July 7 107·4. Fluctuated between 103·8-106·2 till July 15 when it returned to normal.
137	196	T.R. commenced July 4. July 7 106·2. Remained between 104·2-106 till July 18 when it returned to normal.
138	194	T.R. commenced July 4. July 6 106·4. Fluctuated between 103·6-106 till July 12 when it returned to normal.

APPENDIX C II.

THE HISTORY OF FOUR CONTROL SHEEP TO PROVE THE INFECTIVITY OF THE "CONTROL REACTORS" BLOOD USED FOR TESTING THE "IMMUNES" *ex* PASTURE A AND THE "CONTROLS" *ex* PASTURE B. SHEEP INOCULATED SC. WITH 5 C.C. OF "I.R." BLOOD ON 18/7/31.

Ser. No.	*S. No.*	*T.R. commenced.*	*Date and height of first peak.*	*T. in ° F. fluctuated between.*	*T. returned to normal.*
*134	230	July 21	July 22 106·8	102·4-107·2	August 16
*135	223	July 20	July 22 107·6	102-107·4	August 9
139	218	July 20	July 22 108·4	103-106·4	August 16
140	1	July 21	July 22 107·6	103·107·6	August 1

APPENDIX C III.

THE HISTORY OF FOUR CONTROL SHEEP TO PROVE THE INFECTIVITY OF THE "IMMUNE REACTORS" BLOOD USED FOR TESTING THE "IMMUNES" *ex* PASTURE A AND THE "CONTROLS" *ex* PASTURE B. SHEEP INOCULATED SC. WITH 5 C.C. OF "C.R." BLOOD ON 18/7/31.

Ser. No.	*S. No.*	*T.R. commenced.*	*Date and height of first peak.*	*T. in ° F. fluctuated between.*	*T. returned to normal.*
*136	219	July 21	July 22 107	103-106·8	August 1
*137	196	July 21	July 22 108	102·4-107·6	August 2
141	202	July 19	July 20 106·4	102·8-107·8	August 1
142	197	July 21	July 22 107·6	105·6-107	July 29

* Had already been used as controls to prove the infectivity of the virus of "louping-ill." (*Vide* appendix C I.)

Abbreviations.

S.	=	Sheep.
Ser.	=	Serial.
ic.	=	Intracerebral inoculation.
sc.	=	Subcutaneous inoculation.
im.	=	Intramuscular inoculation.
D.B.5.	=	Dried brain 5. A dried and powdered infective brain. Strain of virus Case I 1930 (Greig *et al.* 1931.)
T.	=	Temperature.
N.T.R.	=	No temperature reaction.
V.S.T.R.	=	Very slight temperature reaction.
S.T.R.	=	Slight temperature reaction.
T.R.	=	Temperature reaction.
S.D.	=	Slightly dull.
D.	=	Dull.
V.D.	=	Very dull.
T.L.I.	=	Typical louping-ill.
+X	=	Death x days after inoculation or after being put on to infected pasture.
O	=	No take.
L	=	Lived.
P.M.	=	Post-mortem examination.
C.N.S.	=	Central nervous system.
H.B.	=	Heart blood.
I.R.	=	Immune reactors blood, *i.e.*, infective blood obtained from a louping-ill immune sheep while exposed to infection on infected pasture.
C.R.	=	Control reactors blood, *i.e.*, infective blood obtained from a control sheep while exposed to infection on infected pasture.

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MELANOSIS IN CATTLE.

By Walter Scott, f.r.c.v.s., d.v.s.m.,
Public Health Department, Edinburgh.

Melanosis may be defined as that condition occasionally met with in cattle in which there is an abnormal deposition of dark brown or black insoluble pigment, either generalised or localised in its distribution throughout the body, and which is unaccompanied by any alteration in structure or by any loss of function in the affected organ or tissue ; there are no nodular growths, no metastases, and no alteration in the general metabolism of the affected animal, such as cachexia resulting from intoxication which is frequently associated with malignant neoplasm formation. That it is by no means a common condition met with in the course of routine meat inspection will be evidenced by the following figures : During the years 1928 to 1931, in 28,118 cattle slaughtered in the Corporation Abattoir, Gorgie, Edinburgh, 16 cases of Melanosis were encountered ; in six the pigment was generalised and in ten it was localised. During the same period, 17,627 calves were slaughtered and eight cases of Melanosis were seen—four generalised and four localised (1).

The following is a brief review of some of the cases of Melanosis mentioned in the literature on the subject.

1. Ostertag (2) and Walley (3) mention the occurrence of localised and generalised Melanosis in cattle and the former is of the opinion that the condition is congenital and that it tends to disappear with age. Jaeger (4) is also of the opinion that the condition is congenital.

2. Symmers (5) mentions three cases of generalised Melanosis in cattle which he traced to an enormously thickened layer of pigment behind the retina. One of the animals was known to be blind. The pigment proved to be very insoluble and non-irritating.

3. Perroncito (6) reported two cases—one in an ox and the other in a she goat. There was no appearance of tumour formation and the pigment was granular.

4. Andrieu (7) described a case in the kidneys of a two months old heifer calf.

5. Wooldridge (8) mentions a case in which there were " arborous deposits " in the omentum and on the spleen capsule of a chestnut-coloured bullock.

6. Bailleux and Degive (9) mention a case in a calf.

7. Bru (10) reported a case of generalised Melanosis in a heifer.

8. Mettam (11) described a localised case affecting the lungs, bronchial glands, and liver of a red bullock.

9. Young (12) recorded a generalised case in a red and white cow. This carcase was passed for food, without ill effects.

10. Goldberg (13) mentions a case of localised Melanosis in a calf affecting the membranes of the spinal cord. The cord itself was unpigmented.

11. Sewell (14) reported a generalised case in a three years old red-roan bullock.

12. Cookson (15) mentions two cases—one generalised in a three years old heifer, and the other localised in a six years old cow.

During the last two and a half years the writer has seen 14 cases of Melanosis affecting cattle at the Corporation Abattoir, Edinburgh, and ten of these form the basis of this paper.

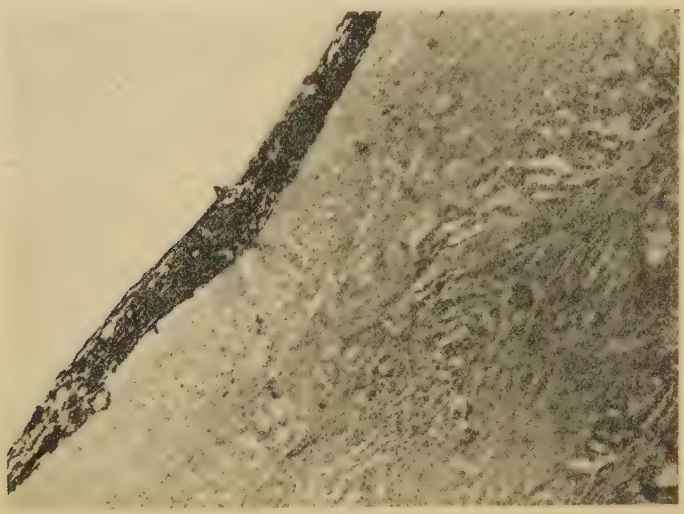

Fig. 1.

From Case 1. × 50. Shows heavy pigmentation of capsule of liver, but none in parenchyma.

Case No. 1.

Subject : A white Shorthorn cross bullock, two and a half to three years old.

Pigmentation was most pronounced in the kidney region, particularly on the right side, and extended backwards into the pelvis and forwards to the third rib. There was no pigmentation of the exterior of the carcase. The adrenals were markedly pigmented ; the kidneys were normal in colour but the fat in this region was black. A pigmented area was seen on the capsule of the liver near the base of the accessory lobe. The trachea was pigmented 6-in. above the bronchial division. The lungs and associated lymph glands showed black areas. Black spots were also present in the mesenteric fat in the pancreatic region. The tongue, hard palate, conjunctiva, and membrana nictitans showed evidence of pigmentation in some degree. The optic nerve appeared normal in colour.

Case No. 2.

Subject : A blue-roan Shorthorn bullock, two and a half years old.

The liver was the only organ affected and pigmentation was confined to the capsule. Several small areas were seen on the diaphragmatic surface of the organ. The hepatic lymph glands appeared normal.

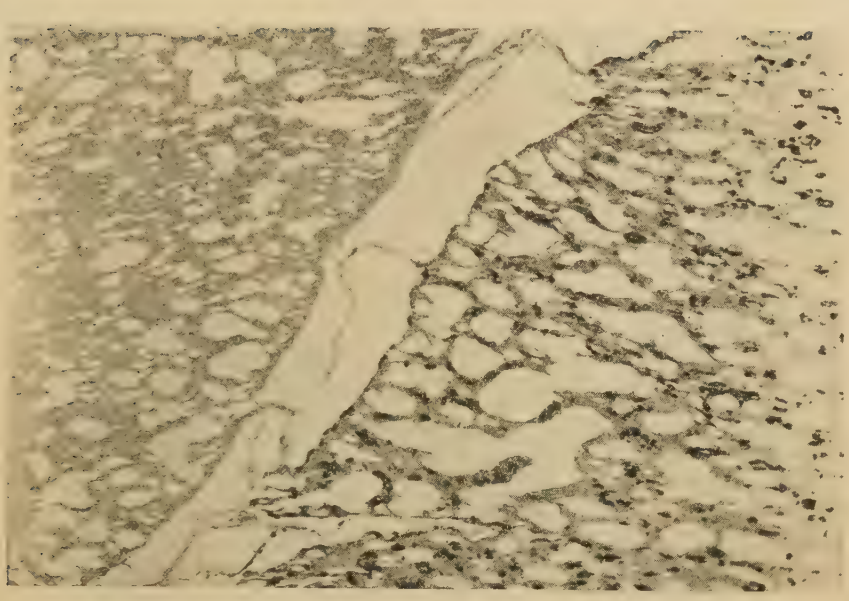

FIG. 2.

From lung of Case V. × 50. Shows distinct line of demarcation between pigmented and unpigmented areas.

Case No. 3.

Subject : A brown and white Shorthorn Cross calf, four months old.

The lungs were the only organs seen to be affected, numerous sharply defined areas being present on the parietal surfaces of both lungs and also throughout their substance.

Case No. 4.

Subject : A black calf, three months old.

Pigmentation was seen extending forwards from the kidney region on both sides of the carcase. The right side was more heavily pigmented than the left. The membranes of the spinal cord were pigmented from the lumbar region to the middle of the thorax.

Case No. 5.

Subject : A brown calf, white in the inguinal region, four months old.

Pigmentation was noted on the parietal surfaces of the lungs and to a less extent throughout the substance. The capsule of the right adrenal showed irregular pigmentation.

Case No. 6.

Subject : A black Polled bullock, two and a half to three years old.

Pigment occurred in spots in the kidney and lumbar region, on the remains of the diaphragm, and in the thoracic region, particularly towards the vertebral column. The membranes of the cord were pigmented throughout their entire length and black spots were seen in the lungs. The capsules of both adrenals were black and spots were noted on the diaphragmatic surface of the liver. In both eyes the conjunctiva was black and the membrana

nictitans was pigmented, particularly towards the free border. Black streaks were seen on the sheath of the optic nerve.

Case No. 7.

Subject : A red Shorthorn bullock, two to two and a half years old.

Pigmentation was seen in the left lung, particularly in the apical lobe. The associated lymph glands were affected and the capsules of both adrenals were pigmented, the pigment being irregular in distribution.

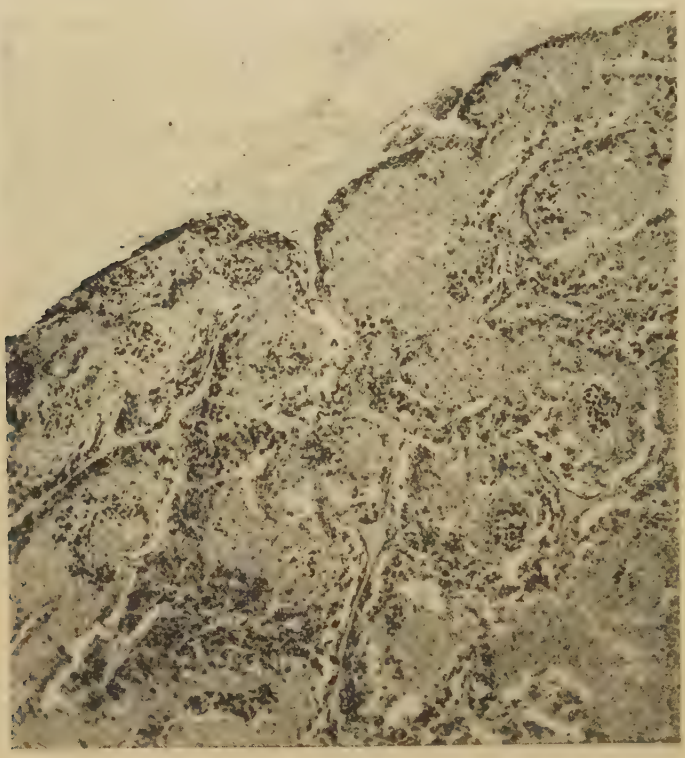

FIG. 3.

From Case 8. × 50. Lymph gland, showing heavy deposit of melanin in the sinuses melanin.

Case No. 8.

Subject : A red Shorthorn cow, aged.

Slight pigmentation was seen on the ribs on the left side. The left apical lobe of the lung was jet black and the lining of the bronchi and trachea was pigmented but the cartilages appeared normal in colour. The associated lymph glands showed streaks of pigment in their substance.

Case No. 9.

Subject : A brown and white Shorthorn bull, about three years old.

The right side of the carcase from the kidney region to the level of the fifth rib was dark grey. The membranes of the cord in this region were

also pigmented. Black spots were present on the capsule of the right adrenal but the left adrenal was unpigmented. The apical lobe of the right lung showed small black areas.

Case No. 10.

Subject : A roan Shorthorn bullock, three years old.

There was heavy pigmentation in the kidney region and this extended into the pelvis and thorax. The remains of the diaphragm was grey, and the right kidney capsule, the right adrenal capsule, and the fat in this region were black. The capsule of the left kidney was spotted. There was no pigmentation of the capsule of the left adrenal, and on section no separation into cortex and medulla could be made out. The organ had a uniform greyish-red appearance and when pressure was applied quite an appreciable amount of blood was expressed. Pigmented areas were present on the surface of the liver. The membranes of the spinal cord from the thoracic region backwards were pigmented and the nerve sheaths leaving the cord were also black. No evidence of pigment was seen on the exterior of the carcase. The diaphragmatic lobe of the right lung was affected but the associated lymph glands appeared normal. The tongue was pigmented throughout its entire length.

Fig. 4.

Ox lungs. From a case not included in the list.

Conclusions.

1. From the Clinical Viewpoint.
2. From the Histological Viewpoint.

Clinical Viewpoint.

1. *Influence of Colour.*—That the condition known as Melanosis does not bear any relationship to the colour of the hide of the

affected animal is indicated by the fact that in the preceding cases the following colours were encountered—white, blue-roan, brown and white, black, red, and roan. In this respect the condition appears to differ from the melanotic new growths which have been most frequently described in veterinary literature in connection with grey horses whose coats are becoming white owing to increasing age. Melanotic neoplasms have been described in cattle but the condition would appear to be rare. Typical tumour formation is invariably absent in true Melanosis.

2. *Influence of Sex.*—The sex of the animal appears to have no particular bearing on the condition since the affected subjects included females, castrated males, and entire animals.

3. *Influence of Age.*—Jaegar (16) in his studies on Melanosis in cattle states that pigmentation is less frequent in full-grown cattle than in calves. The observations made by the writer lend some support to this statement, as in three of the cases melanotic deposits were found in calves of four months and under, and the most pronounced case of Melanosis yet seen by him was in a calf six to eight weeks old. While in this series of ten cases seven were adult animals, it must be remembered that the proportion of adult cattle, compared with calves, slaughtered at the Corporation Abattoir, is seven or eight to one. It is evident, therefore, when the proportion is adjusted, that the condition is more prevalent in calves than in adult cattle.

4. *Influence of Melanosis on the Health of the Affected Animal.*—Under the Public Health (Meat) Regulations (Scotland), 1930, generalised Melanosis is laid down as one of the conditions warranting the total seizure of a carcase. From the point of view of Meat Inspection, it can be readily appreciated that an organ patently affected with Melanosis would raise grave doubts in the mind of the consumer, but it is doubtful if any harm would result from eating such a piece of meat. Young (2) states that the carcase of a cow with generalised Melanosis was passed for food without any ill effects being reported. The examinations carried out in the cases under discussion appear to warrant the expression of the opinion that Melanosis, whether localised or generalised, is a benign condition, because in no instance was there any interference with the health or general well-being of the animal. In every case (with the possible exception of Case No. 8, where the subject was an aged cow in rather poor condition and this poor condition could quite justifiably be attributed to other causes) the carcases were those of well-nourished and healthy animals. In no instance did the post-mortem examination reveal any other extensive pathological condition. In view of the above, and when one bears in mind that the pigment appeared in every case to be non-irritant to the tissues of the affected animal, it appears improbable that harm would result from the ingestion of melanotic tissue.

1. *Histology*.—The pigment was intracellular in the great majority of instances, and where granules were present extracellularly it was in regions where the cells were loaded with pigment. Within the cells there did not seem to be any special part in which the pigment was laid down, but it was frequently noted that when pigment was not present in excessive amounts the granules were gathered around the nucleus and appeared to extend outwards from that region into the cell processes.

2. In the great majority of sections examined the pigment granules were present in increased amounts in the immediate neighbourhood of the smaller blood vessels. Occasionally cells in the interior of the vessels contained granules, but for the most part it was in the cells lying outside the vessels that pigment was present to a marked degree.

3. When the lungs were affected it was found in the majority of sections that the line of demarcation between the pigmented and unpigmented parts was sharply defined. The pulmonary vessels were seen to be surrounded by accumulations of pigment in branching cells and the thin alveolar walls were crowded with small round masses of pigment. Pigment was also seen in the connective tissue of the walls of the bronchial tubes, both internal and external to the muscular layer. Occasionally pigment granules were present in the cells of the corium in bronchial tubes in unpigmented regions of the lungs. In no instance in which small islands of cartilage were examined was any pigmentation of the cartilage cells seen; the condition thus differs from Ochronosis, in which the cartilage cells are pigmented. In the associated lymph glands the distribution of the pigment was irregular; it was found in both cortex and medulla, intra- and extra-cellular, and varied in amount and colour in the different cases.

4. When the liver was affected pigment was present in the capsule and to a less extent in the connective tissue immediately below the capsule. No pigmentation of the actual liver cells was seen and the serous coat of the liver appeared unpigmented. In one case (No. 2) the capsule of the liver was slightly thickened.

5. The membranes of the spinal cord showed a varying degree of pigmentation and occasionally streaks of pigment were seen running into the cord but the cells of the cord were not affected. Similar facts were noted in connection with the nerves.

6. Probably the most interesting point in the whole series of microscopic examinations was in connection with the adrenal glands. It was noted that in the cases of generalised Melanosis the site of heaviest pigmentation was in the kidney and adrenal region. It was also found that the adrenals were affected to a greater or less extent in eight of the ten cases described above. In the other two cases (Nos. 2 and 3) the adrenals were not examined microscopically, and, while there was no evidence of pigmentation in the adrenals

when the carcase was examined post-mortem, it is possible that pigment may have been present microscopically. Pigment was present in the capsule and cortex in varying amounts, but no pigmentation of the medulla was seen. It is true that in some of the cases groups of pigmented cells were seen in the medullary zone, but these cells were always in association with trabeculæ or columns of cortical cells " outcropping " in the medulla. Another point in connection with the adrenals was the fact that pigment did not bear any marked relationship to blood vessels. In one instance (Case 10) there was a departure from normal in the left adrenal. No division into cortex and medulla could be made out. Blood cells were present in large numbers, the vessels and sinuses were engorged, round cells were numerous, but no giant cells were seen.

In connection with the presence of pigment in the adrenals, the following is of interest. Findlay (17) discusses the presence of melanin and lipochrome in the adrenals and states that in human and avian Beri-beri a pigment that is probably melanin is present in them. Again, Goldzieher (17a) discusses pigmentation of the adrenals and states that " these pigments (*i.e.* lipomelanin and lipofuscin) are physiologically active substances which are formed at the boundary of cortex and medulla where the medullary adrenalin may come into close contact with the cortical ferments."

It would appear that melanin production and the adrenals are inter-connected, but at this time no opinion can be expressed as to the nature of this relationship. This connection would appear to be supported by the observation of different workers.

Jaeger (18) found that extracts from a horse melano-sarcoma would oxidise adrenalin to a pigment. Brahns (19) states that melanin causes vaso-constriction. Spencer (20) expresses the opinion that melanin is preceded by a colourless substance which by oxidation becomes a coloured melanin and that it is closely allied to adrenalin. Shaw (21) expresses similar opinions. According to Raper and Wormall (22), oxidation has an important bearing on melanin production. Cameron (23) states that treatment with a strong oxidising agent converts adrenalin into brown and black substances which resemble natural melanin in chemical properties.

The fact that in the present series of cases the pigment frequently occurred in large amounts in the cells around the small blood vessels, where an unlimited supply of oxygen would be available, points in the same direction.

Acknowledgments.

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To my colleagues, Mr. E. J. H. Sewell, M.R.C.V.S., amd Mr. W. Jowett, F.R.C.V.S., I am indebted for assistance in obtaining suitable material, and for advice in connection with the preparation of the sections for microscopic study.

Mr. D. Middleton Greig, of the Royal College of Surgeons, Edinburgh, very kindly allowed me to consult books of reference, periodicals, etc., in the Library of the Royal College of Surgeons, and I am also grateful to Mr. R. Selcraig Murray, Librarian of the Royal (Dick) Veterinary College, for placing certain foreign periodicals at my disposal.

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THE ASSOCIATION OF THE BACILLUS OF PREISZ-NOCARD WITH LESIONS CAUSED BY ŒSOPHAGOSTOMUM COLUMBIANUM IN SHEEP.

By H. R. Carne, B.V.SC.

(*Lecturer in Veterinary Pathology and Bacteriology, University of Sydney*),

and

I. Clunies Ross, D.V.SC.,

(*Parasitologist, Council for Scientific and Industrial Research*).

It has been suggested in Australia that the Preisz-Nocard bacillus may be associated with nodular lesions in the bowel caused by larval forms of *Oesophagostomum columbianum*, the familiar greenish colour of the caseo-purulent material in lesions of both diseases being cited in favour of such an hypothesis. Further, in March, 1930, an officer of the Commonwealth Meat Inspecton Service reported the frequent occurrence of lesions in the ischiatic lymphatic glands in certain mobs of sheep which showed very numerous and prominent nodules of œsophagostomiasis of the rectum. In two cases in which the ischiatic glands were incised in 150 and 200 carcases respectively, 8 per cent. and 10 per cent. were found affected with lesions of what was considered to be caseous lymphadenitis, sometimes both glands being involved. The high percentage of infection of these glands was thought to bear a close relation to the heavy infection of the walls of the rectum by the parasites. Unfortunately all killing of sheep from the particular districts involved had ceased before any material for bacteriological examination could be procured.

It is well known that the larvæ of *Oesophagostomum columbianum* may wander considerable distances from the intestine and are not uncommonly found in such situations as the sublumbar muscles, the lymphatic glands draining the alimentary canal, the liver, and the hepatic lymphatic glands.

The opinion was expressed by one of us (H.R.C.) that the lesions described in the ischiatic glands in these cases were probably due to *Oesophagostomum* larvæ which had wandered from their usual situation of development in the bowel wall. This opinion was based upon a bacterial examination of a considerable number (about 50) of well developed lesions of œsophagostomiasis of the bowel wall of naturally infected sheep, when in only two instances were any organisms detected by cultural and histological methods, and in

both cases these proved to be Gram-negative bacilli of the colon-typhoid group. Also an examination of a small number of aberrant lesions of œsophagostomiasis in livers and lymphatic glands draining the alimentary canal and liver had shown these to be sterile in every case.

One of us (H.R.C.) has also found that greenish coloration of pus in sheep is not a specific character of lesions of the Preisz-Nocard bacillus, but is also found commonly in suppurative conditions of sheep caused by other organisms.

Certain European workers claim that ingestion is the common method of infection of sheep by the bacillus of Preisz-Nocard, and Carré (1928) attributes to intestinal parasites a very important rôle in facilitating this infection by producing injuries of the mucosa of the alimentary canal which act as portals of entry for the Preisz-Nocard bacilli. This view is definitely opposed to that of most Australian investigators, who consider that the usual method of infection is through contamination of wounds of the skin.

In view of the importance of determining whether infection with the Preisz-Nocard bacillus does occur from such an association with intestinal parasites, more particularly with *Oesophagostomum columbianum*, it was decided to carry out an experiment in which sheep were to be drenched with cultures of the Preisz-Nocard bacillus and simultaneously infected with *Oesophagostomum* larvæ, thus affording optimum conditions for the claimed associated lesions to develop.

Experimental Animals Used.

Nine lambs four months old which were unshorn but had been tailed and castrated and had come from one property. The majority were apparently pure Merinos but one or two had evidence of some long-wool blood. Throughout the experiment, which lasted 51 days, all the animals were run together in a yard with bricked floor and were fed on oaten chaff to which a quantity of mineral mixture and bran was added, together with a fairly liberal supply of lucerne hay.

Experiment.

Seven lambs were drenched with 10^{-2} gm. of moist Preisz-Nocard bacilli suspended in 10 c.c. of sterile physiological saline, followed by 150 c.c. of tap water. This dose was repeated the following day. Several hours after the second dose five of these sheep and two others which had not received culture were each drenched with approximately 1,500 larvæ of *Oesphagostomum columbianum* (together with some 6,000 trichostrongyl larvæ). Thirteen days later three of the experimental sheep and one culture control received a further massive dose of 4×10^{-2} gm. of Preisz-Nocard culture. The three experimental sheep receiving this dose were then drenched with another 500 *Oesophagostomum* larvæ. The details of the amounts of culture and larvæ received by each sheep are shown in Table 1.

TABLE I.

Sheep No.	*Preisz-Nocard Culture.*			Total.	*Oesophagostomum Larvæ.*		Total.
	15/9/30	16/9/30	29/9/30		16/9/30	29/9/30	
	gm.	gm.	gm.	gm.			
70	10^{-2}	10^{-2}	4×10^{-2}	6×10^{-2}	1,500	500	2,000
67	10^{-2}	10^{-2}	4×10^{-2}	6×10^{-2}	1,500	500	2,000
65	10^{-2}	10^{-2}	4×10^{-2}	6×10^{-2}	1,500	500	2,000
64	10^{-2}	10^{-2}	—	2×10^{-2}	1,500	—	1,500
68	10^{-2}	10^{-2}	—	2×10^{-2}	1,500	—	1,500
66	10^{-2}	10^{-2}	4×10^{-2}	6×10^{-2}	—	—	—
63	10^{-2}	10^{-2}	—	2×10^{-2}	—	—	—
69	—	—	—	—	1,500	—	1,500
71	—	—	—	—	1,500	—	1,500

Preparation of Culture for Drenching.

Freshly prepared serum peptic digest agar bottles were sown with a 24 hours serum broth culture. Bottles were slightly tilted during incubation in order to prevent the water of condensation from running over the surface. After 48 hours incubation at 37° C. the luxuriant growth was washed off with sterile saline. The culture from each bottle was examined for purity and then centrifuged to throw the organisms to the bottom. The moist deposits were then transferred to a sterile Petri dish containing a disc of sterile filter paper. After the excess moisture had become soaked up, one gram of the moist bacterial deposit was emulsified in 100 c.c. of sterile physiological saline by placing in a shaking machine for one hour. The initial dose of 10^{-2} gm. was prepared by taking 1·0 c.c. of this suspension and, for greater certainty in administration, diluting it further with 9 c.c. of saline. The sheep were drenched by means of a syringe without its needle.

The pathogenicity of this strain of the Preisz-Nocard bacillus (B 57) had been determined in 1929 by inoculating sheep by various routes with graded doses of washed serum agar culture. It was found that a dose of 10^{-2} gm. inoculated intravenously killed a full-grown sheep in 24 hours, whereas the same dose given subcutaneously was fatal in 72 hours. In order to ascertain whether the pathogenicity of the strain had become altered during artificial cultivation, two full-grown sheep were inoculated with 10^{-2} gm., one subcutaneously in the thigh and the other intravenously. These inoculations were made on the same day that the sheep in the experiment received their first drench, and the same emulsion was employed. The sheep which received the intravenous inoculation became rapidly emaciated and developed severe lameness in two limbs within several days following inoculation; they remained in a very emaciated condition for the duration of the experiment, but did not die.

The sheep which was inoculated subcutaneously developed a marked local lesion and severe lameness within 24 hours, but the

lesion became chronic and the lameness later disappeared. This animal also remained alive throughout the experiment.

It was thus seen that this strain of the bacillus had lost a considerable degree of its virulence but was still capable of producing severe lesions in the dose employed in the experiment, *viz.*, 10^{-2} gm., when inoculated subcutaneously and intravenously. It was because of this decreased virulence and the fact that all of the sheep appeared o have tolerated the doses already given that it was decided to give a third and larger dose to certain animals as indicated above.

In order to gain some idea of the number of organisms contained in the doses of suspension given, dilutions were made and plated out on serum agar. The results of this estimation would indicate that sheep which were drenched with two doses of suspension received approximately 2 million bacilli, and those which received the third larger dose a total of approximately five million. These figures can only be taken as minimal because the tendency of the Preisz-Nocard bacillus to grow in closely adhering clumps is a well known characteristic, and it is impossible to obtain disintegration of these clumps when cultures from solid media are suspended in saline. Hence the plating out of dilutions is at best a very inaccurate method of counting, and the results thus gained will probably have to be multiplied by anything up to 10 or even more in order to obtain the correct number of organisms in the emulsion.

The doses given, however, have been quite liberal when compared with the virulence of the organism administered by other routes.

The first two sheep were killed 37 days after the commencement of the experiment, and the remainder at intervals up to the fifty-first day.

In carrying out post-mortem examinations, the lymphatic glands throughout the body were submitted to detailed examination; pulp was taken from about 30 of the most important of these in each animal and sown on serum agar. The glands were then sliced finely in order to detect any macroscopic changes which might not have been visible externally. In almost every case glands which showed lesions were also examined histologically.

Bacteriological examination was also made of the contents of ten *Oesophagostomum* nodules from each case. The material for examination was obtained by searing the peritoneal surface of the bowel over the lesion, which was then opened with a sterile knife. The greater part of the contents were then removed and transferred to serum agar plates, over which they were spread as evenly as possible by means of a sterile bent glass rod.

Parasitological examination included determination of the numbers and species of parasites present in the whole alimentary tract, detailed examination of the nodules for the presence of fourth stage larvæ of *O. columbianum*, determination of the presence or absence of immature forms in the mucus of the intestinal wall, and

enumeration and determination of the sex of mature adult *Oesophagostomum columbianum* in the intestinal contents.

General Results of Post-mortem Examinations.

Abomasum : all sheep showed some degree of infestation by *Hæmonchus contortus* and *Trichostrongylus* and *Ostertagia spp.*

Small Intestines : four sheep (64, 67, 68, 69) showed numerous young *Oesophagostomum* nodules scattered throughout their length. Five were free from lesions, and in this number were included both the culture controls which had not received larvæ.

Large Intestines : typical *Oesophagostomum* nodules were sprinkled throughout except in sheep 63 (culture alone control), which was entirely free. The nodules varied from about 50 (in the second culture alone control) to many hundreds. They were usually much more numerous and prominent in the last few feet of the colon and rectum. The contents of nodules were of a greenish colour, varying somewhat in brightness of tint, being brightest in the youngest lesions. Their consistence varied from a soft, creamy, purulent, to firm caseous material. Scattered amongst the young fresh lesions in some animals were a few old and some calcified nodules resulting from a natural infection prior to the experiment. In most cases a proportion of the well developed nodules had ruptured into the lumen of the bowel, and in some cases the contents of the abscesses together with small quantities of blood and mucus were present on the surface of the fæces in the lower part of the colon. The majority of young nodules, however, were closed.

In most cases a variable number of mature adult male and female *Oesophagostomum columbianum* were present in the contents of the large bowel. In some animals immature adult forms were also present, not yet having completed their development after escaping from the nodules. Examination of the contents of nodules showed that in all but Sheep 66 a considerable percentage contained fourth stage larvæ (24 per cent.–60 per cent.).

Scrapings made from the mucous membrane of the large bowel demonstrated the presence of fourth stage larvæ in this situation in a number of animals.

Lesions in Lymphatic Glands.

Since lesions of caseous lymphadenitis are most commonly found in lymphatic glands, all the most important glands of the body have been included in the examination. Particular attention has been paid to lesions occurring in lymph glands draining the alimentary tract, and more especially the intestines. As noted above, larvæ of *Oesophagostomum columbianum* may wander to situations other than their usual seats of predilection in the bowel wall and are not uncommonly found in the lymphatic glands draining the large bowel. That fact has been amply confirmed by this experiment, in which at least 12 glands draining the intestines have been found

to contain lesions caused by wandering larvæ, and lesions of the same nature were also found in the livers of three animals.

Apart from the negative findings when pulp from such lesions was sown on appropriate culture media, it has been found possible to distinguish readily between lesions caused by larval *Oesophagostomum columbianum* and the Preisz-Nocard bacillus by means of the characteristic histological picture in each case.

Discussion.

In all the sheep a heavy infection of the bowel wall with *Oesophagostomum columbianum* larvæ was produced by the larvæ administered. In addition, considerable numbers of other intestinal parasites were present (*Hæmonchus contortus*, *Trichostrongylus spp.*, *Ostertargia spp.*). The dose of Preisz-Nocard bacilli ingested was far greater than would be likely to occur under natural conditions.

If injuries to the intestinal mucosa caused by intestinal parasites are common portals of entry of the Preisz-Nocard bacillus, optimum conditions for the development of lesions of caseous lymphadenitis were present in these experimental animals. Infection of lymphatic glands with the Preisz-Nocard bacillus, however, occurred in only three of the test sheep, and only in one of these were lesions in a gland draining the intestinal tract in which the injuries caused by the parasites were present. In the other sheep lesions were confined to the submaxillary glands. The fact that the sheep used in the experiment were lambs, that they had not been shorn, that early lesions of caseous lymphadenitis were found in submaxillary lymphatic glands in three animals, and that control animals were free from lesions of caseous lymphadenitis, is strong evidence that the lesions found in these sheep were the result of infection by the organisms administered *per os*, and not from a natural infection prior to the experiment. As these sheep were about three months old, they were due to be cutting their fourth molar teeth, and it is possible that the occurrence of lesions of the submaxillary lymphatic glands may be referable to this circumstance.

In Sheep 68 Preisz-Nocard bacilli were recovered from the right prescapular and one mesenteric lymph gland, both submaxillary glands being normal. This case is of particular interest because of the situation of the lesions. The pipette of pulp from the affected mesenteric gland failed to show any growth when sown on appropriate culture medium, but when the gland was incised two greenish abscess areas were present. The consistence of the contents of one of these appeared somewhat softer than the other lesions caused by *Oesophagostomum columbianum* in lymph glands of the same sheep. A loopful of the material was therefore sown on serum agar, in spite of the fact that the gland had not been opened aseptically. By misadventure this gland was not kept for histological examination. The heavy growth of pure Preisz-Nocard bacilli which was obtained left little doubt that the lesion was due to that organism. It is

worthy of note that there were lesions in two other mesenteric and two colic lymph glands in this sheep which macroscopically were practically identical in appearance but gave negative cultural results and were all definitely lesions of œsophagostomiasis histologically.

In this case very numerous young *Oesphagostomum* nodules were present in both small and large intestines, so that if these lesions offer a portal of entry to the Preisz-Nocard bacilli there was ample opportunity for this to take place. This sheep received the smaller dose of culture, *i.e.* 2×10^{-2} gm.

The lesion in the right prescapular gland might be explained in several ways. Firstly, it may have been a naturally contracted infection from some skin wound in an area drained by the prescapular gland. In the second place it may have been secondary to the lesion in the mesenteric gland, the organism having reached the general circulation *via* the lymphatics draining to the thoracic duct from the primary lesion. If organisms were carried free in the lymph stream, one would have expected to have found secondary lesions in the lungs (which are a common site of secondary lesions of caseous lymphadenitis). It has been observed by a number of workers, however, that infection experiments are sometimes followed by development of primary lesions in lymph glands remote from the site of introduction of the bacilli ; this applies more particularly to animals receiving culture by the mouth. The only satisfactory explanation that has been put forward to account for such results is that of L. B. Bull (1929), who suggested that the bacilli may be engulfed by wandering phagocytes, which might then be carried by the lymph and blood streams for some time before succumbing to the effects of the ingested bacilli.

The evidence in favour of the lesions in this prescapular gland being secondary is that the lesion was quite an early one, that the animal had not been shorn, and that it did not show any noticeable skin wounds.

Another possibility to be considered is that the bacilli may have been taken up by wandering phagocytes and carried through the bowel wall quite apart from the primary lesion, and eventually been carried by the vascular system to the lymph gland before succumbing to the toxins produced by the ingested organisms. It is generally found, however, that if Preisz-Nocard bacilli gain entrance to a wound a local lesion results, or lesions develop in the nearest group of lymph glands draining the site of infection. So that if infection by the Preisz-Nocard bacillus of injuries of the intestinal mucosa caused by *Oesophagostomum columbianum* or other helminth parasites were a common occurrence, it is reasonable to assume that lesions of caseous lymphadenitis in lymphatic glands draining the intestinal tract would be commonly observed. From the extensive data concerning the occurrence of lesions of caseous lymphadenitis of sheep which recently have been accumulated in Australia by the Commonwealth Meat Inspection Service and others, an outstanding

fact is the rarity of primary lesions of glands draining the intestinal tract, whereas parasitic infection of the alimentary tract and lesions of caseous lymphadenitis in lymphatic glands draining the superficial areas of the body are very prevalent in sheep in Australia.

The experiment has also produced further evidence that lesions in lymphatic glands draining the intestines may be caused by wandering larvæ of *Oesophagostomum columbianum*, and it is interesting to note that 12 abdominal lymph glands were found to be thus affected, whilst only one showed lesions due to the Preisz-Nocard bacillus. A point of practical importance is the fact that the macroscopic appearance of early lesions of Oesophagostomiasis and infection by the Preisz-Nocard bacillus in lymph glands are very similar and may quite pardonably be mistaken on ordinary post-mortem inspection.

From the findings in this experiment we are of the opinion that injuries of the intestinal mucosa caused by the helminth parasites, more particularly by *Oesophagostomum columbianum*, are not a common portal of entry of the Preisz-Nocard bacillus under natural conditions. This opinion supports the post-mortem observations at slaughter-houses of the rarity of primary lesions of caseous lymphadenitis in the abdominal lymphatic glands of sheep in Australia.

Summary.

1. Five lambs have been simultaneously drenched with cultures of Preisz-Nocard bacilli and larvæ of *Oesophagostomum columbianum*.

2. In spite of marked lesions of Oesophagostomiasis of the bowel wall and the presence of considerable numbers of other intestinal nematodes, lesions caused by the Preisz-Nocard bacillus only occurred in one mesenteric gland in one animal (which was also affected in the right prescapular gland) though infection of the submaxillary lymph glands occurred in three animals.

3. Lesions caused by wandering *Oesophagostomum* larvæ were present in 12 abdominal lymph glands and three livers. These lesions were free from bacteria.

4. The histological pictures in lesions of *Oesophagostomum columbianum* and *Preisz-Nocard bacilli* in lymph glands are characteristic and readily distinguished from each other.

5. Bacteriological examination of *Oesophagostomum* nodules from the bowel wall failed to reveal the presence of the *Preisz-Nocard bacillus*, though other bacteria were recovered from a proportion of the nodules.

6. The authors are of the opinion that injuries of the intestinal mucosa caused by intestinal parasites, more particularly by *Oesophagostomum columbianum*, are not a common portal of entry of the *Preisz-Nocard bacillus* under natural conditions.

EPIZOOTIC LYMPHANGITIS: MYCELIAL FORMS OF THE PARASITE IN A NATURAL CASE.

By S. C. J. Bennett, B.SC., M.R.C.V.S.,

Veterinary Research Officer, Sudan Government.

The object of this short paper is to place on record the observation of mycelial forms of *Cryptococcus farciminosus* in a naturally occurring case of epizootic lymphangitis.

Apart from the discovery of these mycelial forms the case itself was of some interest, in that it was one of generalised infection, such as is rarely seen; in fact, except in a country served by a very scattered veterinary staff, a case could hardly arrive at such an advanced stage of the disease.

The patient was an aged horse owned by a native in the Blue Nile Province of the Sudan. When seen by the Province Veterinary Inspector (Capt. L. E. Prichard, O.B.E., who provided the material now under discussion), practically the whole of the horse's body was covered with open cutaneous lesions. These were so diffuse, or so close together, that little cording of the cutaneous lymphatic vessels could be seen. In addition, there was a glue-like yellowish discharge from both nostrils and the submaxillary lymphatic glands were enlarged. The general appearance, indeed, was such as would not have been at variance with a very advanced case of glanders, although that disease has never yet been found in the Sudan. The horse was destroyed, and the post-mortem examination revealed a number of pea-like nodules scattered throughout the lungs, thus still more closely simulating glanders. The entire head and smears (only) from lung nodules were sent to the laboratory.

Examination of the head revealed extensive infection of both the skin and the nasal mucous membrane, the latter showing in both nostrils considerable confluent irregular areas of suppurative ulceration. In all sites, typical cryptococci could be demonstrated in large numbers.

The particular interest, however, lay in the smears from the lung lesions. These, although containing numbers of typical cryptococci, were relatively much richer in mycelial elements exactly similar to those which the organism produces in artificial culture media. The relative richness of "typical" and mycelial forms is seen in Fig. 1, which is a photograph of an area selected almost at random at a moderate magnification. Figs. 2 and 3 show other portions of a smear under progressively higher magnifications; in both cases, fields containing a few "typical" cryptococci are selected in order that the relative sizes of the cryptococcal and mycelial elements may be appreciated. It may be further noted, although the fact is not evident from the microphotographs, that no tissue cells of any kind could be detected in suitably-stained material.

FIG. 1.
× 150.

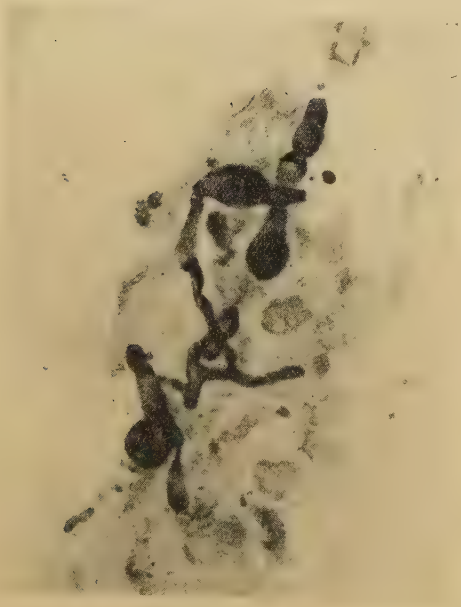

FIG. 2.
× 650.

A brief note concerning the staining capacity of the various elements may also be made. The difficulty of obtaining reasonably detailed pictures of cryptococci is well known, but the preparations at present under consideration were more than usually difficult. The

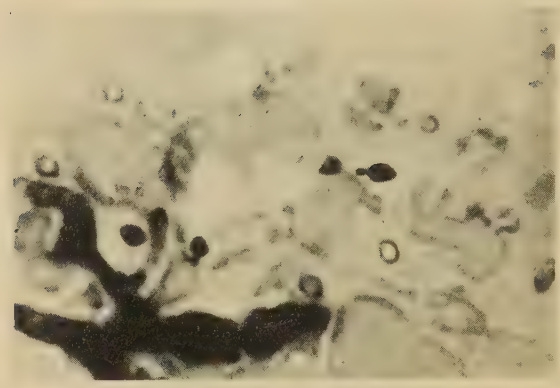

FIG. 3.

× 1,200.

mycelial elements, although intensely " fast " to Claudius' modification of Gram's stain, were much more easily decolourised by Weigert's modification than the " typical " elements. Ultimately, for the taking of microphotographs, the stained smears were differentiated with a dilute solution of aniline in xylene and finally rinsed in the latter. Even so, however, ideal preparations could not be obtained, as can be seen in Figs. 2 and 3, in which the differentiation is markedly unequal.

It is unfortunate that one was unable to submit a complete lung nodule to histological examination, since it would have been of interest to determine the exact process by which the contents were cut off from the surrounding lung tissue. *A priori* one would expect that a capsule of fibrous tissue would have been present, but in an earlier study of pulmonary cryptococcus infections* a special note was made of the strong inhibition of fibrous tissue formation. It is fairly certain, however, that by some process the contents of the nodules were completely cut off from the surrounding tissue, and such isolation would reasonably explain the development of forms of the parasite that do not normally occur in living animal tissue.

In any case, as far as can be ascertained from the literature,† mycelial elements of *C. farciminosus* have been regarded as entirely characteristic of artificial cultures and have hitherto never been reported as occurring in a living animal; for this reason, therefore, the present case is worthy of being placed on record.

Note.—The figures are from microphotographs by Mr. A. Marshall, Wellcome Tropical Research Laboratories, Khartoum, by courtesy of the Director (Major R. G. Archibald, C.M.G., D.S.O.).

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* See this Journal, 1931, Vol. xliv, pp. 85-105.

† Note by Editor. Short mycelial filaments in pus from the lesions were described by Tokishige in Japan.

SALMONELLA INFECTIONS IN DUCKLINGS.

By Norman Hole.

Veterinary Laboratory, Ministry of Agriculture and Fisheries.

Much literature has been published on the relationship of salmonella infections in animals to food-poisoning in man. The subject of such infections in birds, however, is one of peculiar interest, in view of recent statements connecting eggs, particularly duck eggs, with outbreaks of food-poisoning. Scott (1) describes three and mentions seven outbreaks in which he considers that the circumstantial evidence points so strongly to duck eggs being the source of *B. ærtrycke* infection that no other proof is required. Such a conclusion may satisfy epidemiological requirements, but bacteriologically we must have positive proof of the occurrence of the organism in the egg. It is well known that one member of the salmonella group, *B. pullorum*, can be isolated from the egg, and Baudet, quoted by v. Heelsbergen (2) describes the egg transmission of a *B. paratyphosus* B type in the fowl. There is a tendency to assume that other members of this group causing disease in birds may be transmitted to the eggs by adult carriers, but no other organism has definitely been shown to be passed to the egg in this manner.

Salmonella infections are not rare in birds, even if one excludes *B. pullorum* and *B. sanguinarum*, which organisms are not regarded by some as true members of the group. Epizootics caused by *B. ærtrycke* have been described in canaries (3), (6) and parrots (4), in pigeons (5), in chicks (7), (8), (9), adult hens (8), in geese (13) and in ducklings (10). *B. enteritidis* has been found in ducks (8), chicks (8) and pigeons (2) whilst de Jong(11) mentions a *B. paratyphosus* B infection of sparrows described by Tartatowsky, and has himself encountered the same organism in the partridge and fowl. Owing to its close association with *B. ærtrycke*, *B. anatum* must also be considered. It has been described affecting ducklings in conjunction with *B. ærtrycke* (10), affecting chicks in conjunction with *B. ærtrycke* (9), and affecting ducklings alone (12). This latter account of Keel disease, the only one hitherto described in England, is the subject of further consideration in this article.

It would seem, therefore, that many varieties of birds are susceptible to salmonella infections, and it is obvious that the eating of infected flesh or tissues might cause food poisoning in man. This, indeed, has been observed in Germany, due to eating the liver or smoked breast of the goose (14). Several authors have isolated the organisms from the ovaries of affected birds, but none have obtained cultures from the eggs, and there is, therefore, no bacteriological support, at present, for Scott's theory (1) that ducks' eggs may be infected from the ovary. It must be remembered, however, that only a percentage of the eggs laid by a *B. pullorum* carrier hen are infected, and a large number of eggs would have to be examined before such negative evidence can be accepted.

Personal Observations.

In the course of routine diagnosis work, three epizootics in ducklings have been encountered—two in 1930 and one in 1931. In each case it was a salmonella infection, one being due to *B. enteritidis*, the other two to ærtrycke types. Farm B, where *B. enteritidis* was concerned, was the subject of considerable investigation, and a number of the adult stock were brought and examined over a period at the laboratory. On Farm F and Farm S, however, no further investigations were made. The three farms were situated in widely separated parts of the country.

Farm B. (Organism " B.")

This owner wrote saying that, after five years of successful duck rearing, heavy losses were occurring in the present season ; both hatching and rearing results were poor. No symptoms were described, it being merely stated that the ducklings usually died within 10 days of hatching. Specimens were forwarded, and bacteriological examination resulted in an organism being obtained from the livers of each case in pure culture.

Farm F. (Organism " F.")

A number of dead ducklings were received, about ten days old. The only information that could be obtained was that numbers were dying off. Pure cultures were obtained from the livers of the specimens.

Farm S. (Organism " S.")

This was a Farm Institute. Apparently only one batch of ducklings was affected. A number died, and pure cultures were obtained from the livers of the specimens forwarded. A recommendation to kill the remainder and carry out thorough disinfection was adopted with apparent success.

It was possible to examine the adult stock only on Farm B.

The Organisms.

In primary cultures all three organisms grew readily on agar-agar and in peptone broth. The agar slopes showed a heavy growth rather like *B. coli* ; the broths were uniformly turbid, showing a much heavier growth than *B. pullorum*. All cultures proved to be Gram-negative pleomorphic bacilli, and did not ferment lactose.

The following cultures were obtained for comparative work. From the National Institute of Type Cultures strains of *B. enteritidis* 75, *B. anatum C*1, *B. anatum C*5, *B. anatum* 850 (ærtrycke type) and *B. anatum* 851 (ærtrycke type) ; by courtesy of Professor Gaiger a culture of the organism described by Gaiger and Davies (12). In addition two stock strains of *B. pullorum* (22 and 26a) and a stock strain of *B. ærtrycke* (originally obtained from Dr. Bruce White) were used. Before commencing any tests all cultures were plated and subs made from single colonies.

Morphologically it was not possible to differentiate any of the cultures. All except *B. pullorum* were motile. Culturally *B. pullorum* was distinguished by its more delicate growth, and *B. anatum C*1 and *C*5 by their heavy growth in peptone broth. All cultures grew in, but none liquefied, gelatin.

Biochemical Reactions.

The organisms examined fell into three groups :—

Group I. *B. enteritidis* 75, *B. anatum C*1 *and C*5, *B. anatum* (Gaiger and Davies), Organism " B " and Organism " S," all produced acid and gas in glucose, mannite, dulcite, arabinose, maltose, sorbite, dextrin, rhamnose, galactose, xylose and lævulose ; they grew, but without acid production, in lactose, saccharose, raffinose, adonite, inulin, salicin and inosite. Litmus milk showed little change after 24 hours at 37° C., but later became markedly alkaline. Indol was not formed, but nitrates and lead acetate media were reduced.

Group II. *B. ærtrycke*, *B. anatum* 850 and 851, and Organism " F " differed from Group I only in that acid and gas were produced in inosite.

Group III. Both strains of *B. pullorum* produced acid but no gas in glucose, mannite, arabinose, galactose and lævulose. One fermented rhamnose and the other did not. Litmus milk tended to turn acid. Nitrates were not reduced.

Remarks.

Throughout these biochemical tests Andrade's indicator was used, except with maltose, to which methyl red was added after incubation. Tests were repeated several times, but reactions remained constant. Gaiger and Davies (12) employed the method of Christiansen and used bromthymol blue as an indicator. Their results do not completely agree with those here recorded, the most important difference being in inosite ; they found this substance irregular in its reaction, and did not distinguish *B. anatum* ærtrycke types from non-ærtrycke types. We found inosite reactions constant, although sometimes showing no reaction until the forty-eighth hour. (All tests were examined daily for five days). Some media were made up with bromthymol blue indicator, and although no definite differences were found in fermentation results such a wide range of colour between blue green and yellow occurred in uninoculated control tubes that the reading of results was very difficult. This, however, may conceivably have been due to faulty technique in preparation.

The two *B. pullorum* strains are included for the purpose of contrast, for *B. pullorum* and *B. enteritidis* are closely related serologically. In the course of routine examinations for pullorum disease of a large number of chicks at this laboratory, cultures have occasionally been isolated which probably belong to the ærtrycke or enteritidis groups. Unfortunately, pressure of work did not permit their further investigation, but, even if they were agglutinated by pullorum serum, they were readily differentiated by their greater fermentative ability and their plentiful gas production. Although *B. pullorum* is generally

classed as a gas producer, in the writer's opinion gas production is rare, whether the organism is isolated from chick or adult hen, at least, in primary cultures.

Serological Reactions.

The serology of these organisms was only examined superficially ; no attempt was made to decide their exact antigenic structure. Simple antisera were prepared by inoculating rabbits intravenously at weekly intervals with 24-hour broth cultures killed by heating at 56° C. for 15 minutes. The initial dose was 0·2 c.c., raised to 1 c.c. by 0·2 c.c. stages ; the animal was then bled out, a titre of about 1,000 having been attained. Antisera were prepared in this manner against *B. enteritidis*, *B. anatum C*1, *B. ærtrycke*, *B. pullorum* (22 and 26 mixed), *B. anatum* (Gaiger and Davies), Organism " B " and Organism " F." Organism " S " was isolated much later, and no antiserum prepared. The antigens used throughout were carbol saline washings of 24-hour agar cultures, made up to a standard density. Control tests were always run in parallel with a negative rabbit serum. All agglutinations were macroscopic tests incubated for 18 to 24 hours at 37° C.

Preliminary agglutinations divided the organisms into three serological groups.

Group I. *B. enteritidis*, *B. anatum* (Gaiger and Davies), Organisms " B " and *B. pullorum*, all cross agglutinated in dilutions of 1 : 500 and over.

Group II. *B. ærtrycke* and Organism " F " also cross agglutinated, and both of these sera agglutinated *B. anatum* 850 and Organism " S."

Group III. *B. anatum C*1 agglutinated itself and *B. anatum C*5 to a high titre.

Inter-group agglutinations. Some agglutination occurred between *B. anatum* (Gaiger and Davies) and *B. anatum C*1 and *C*5. It was only partial, however, even in 1 : 50 dilution. *B. anatum C*1 showed a similar slight reaction with *B. anatum* 850 and, to a lesser degree, with *B. ærtrycke*. No other inter-group agglutination were observed. The dilutions used ranged from 1 : 50 to 1 : 500.

Absorption tests were carried out with organisms of the Groups I and II. Dense saline emulsions were used with serum diluted 10 times and the absorptions repeated until a test agglutination with the homologous antigen showed that all or nearly all the agglutinins had been removed.

The results are shown in Tables I and II.

Serological conclusions. It is not suggested that the serological investigations are complete. The antigens and antisera used, however, should contain both " H " and " O " components, and should admit conclusions as to the close affinities of the organisms examined.

It will be noted that in Group I, *B. enteritidis*, *B. anatum* (Gaiger and Davies) and Organism " B " appear to be identical ; *B. pullorum*

drops out. Group II results are not quite so clear ; *B. ærtrycke* and Organism " F " appear to be the same, but the introduction of *B. anatum* 850 antigen conflicts the result. With regard to Organism " S," it can only be stated that it appears to contain all the antigenic components of *B. ærtrycke* ; it may contain more.

The results obtained are about what would be expected if one examines an antigenic table drawn up by Bruce White (15). *B. pullorum* has only a somatic antigen, which is the same as that of *B. enteritidis*. It does not absorb any of the " flagellar " antibodies, however, which are left to react with *B. enteritidis*. *B. enteritidis* shares no antigenic constituents with *B. ærtrycke*, so no degree of cross agglutination would be expected. *B. anatum* is not considered in the table, but it is conceivable that its antigenic structure has something in common with *B. ærtrycke* on one hand and something else in common with *B. enteritidis* on the other. It is interesting to note that Gaiger and Davies (12) compared their organism to *B. ærtrycke*, *B. paratyphosus A*, and *B. paratyphosus B*. They obtained no agglutination in a dilution of 1 : 20. If their organism is really an enteritidis type, as here stated, this result would be expected, for Bruce White's table shows the organisms mentioned to have no antigenic component common with those of *B. enteritidis*.

Pathogenicity of Organisms " B " and " F."

The effect of both these organisms was tested on ducklings, chicks, adult fowls, rabbits, and guinea-pigs. Organism " F " proved the more pathogenic for chicks and ducklings under experimental conditions. Indeed, it seemed doubtful whether Organism "B " was pathogenic for ducklings of any age if the birds were strong and kept under good conditions. Gaiger and Davies (12) also failed to infect 10 days old chicks and three weeks old ducklings with their organism. A comparison of the two organisms is shown in Table III.

Further Investigations.

Farm " B " was the only farm where any attempt was made to trace the source of infection. It was situated in the West of England, in woodlands. The general conditions were quite good, except that all buildings were over-run with vermin. The adult duck stock appeared to be very healthy and were laying well. Two sources of infection were considered ; vermin are known to suffer from epizootics due to salmonella organisms, and it was quite conceivable that they were infecting the food ; some of the adult ducks might be carriers of infection, and be passing it on to the ducklings through the egg. The question of vermin was discussed with the owner, who promised to make every effort to eradicate it and forward us any dead specimens. No specimens have been received, and so no conclusions can be drawn. No rat virus had been used on the farm.

The possibility of egg transmission was examined under the following headings :—

1. Blood samples taken from a number of ducks that had produced the hatching egg concerned were tested for agglutinins.

2. Thirty-three ducks, some of which gave a suspicious reaction to the blood test, were bought and kept at the laboratory.

3. The eggs of these ducks were saved ; a number were hatched out in an incubator and the remainder were examined for bacterial infection.

4. These ducks were killed and post-mortem and bacteriological examinations made.

The Agglutinating Properties of Blood from Adult Ducks.

Serum was separated from the blood and tested against antigens of Organism " B " and *B. pullorum*. A number of duck bloods from a farm where disease was not known to have occurred were tested at the same time. Duck 194, from Farm " B," completely agglutinated both antigens in 1 : 50 dilutions. A number gave distinct agglutinations in 1 : 20 dilutions, but so did a number of samples from presumably healthy ducks.

It was also noted that the agglutination with *B. pullorum* antigen was often the more marked. So little is known about the presence of normal agglutinins in the blood of ducks that no conclusions can be drawn from a 1 : 20 reaction. It was unfortunate that repeated requests failed to obtain Duck 194, which appeared to have been lost. Further blood tests at the laboratory on the 33 live ducks obtained gave very similar results, and in no case was a reaction obtained in dilutions higher than 1 : 20.

Hatching Results.

The conditions under which the ducks were kept at the laboratory were not ideal for the production of fertile eggs. Nevertheless, of 90 eggs set, 59 proved fertile, although 23 of these were dead in shell. Of the 36 ducklings hatched, eight were weaklings and died by the fourth day ; the remainder thrived and were in excellent condition at three months old. Twelve infertile eggs, 12 dead in shell, and the eight ducklings that died were all subjected to bacteriological examination. Cultures in every case proved sterile.

Egg Culture Experiments.

All cracked eggs were discarded. Sound eggs not required for hatching were kept in the incubator for three to four days at 37° C. They were then immersed in absolute alcohol for one minute, the shell cracked with sterile forceps and 2 c.c. of yolk sucked up in a pipette and inoculated into 20 c.c. of peptone broth. After 24 hours incubation at 37° C., a loopful of this broth was re-inoculated into fresh broth and examined for growth the next day.

Over a two-month period 100 eggs were examined in this manner. In two cases a growth was obtained which proved to be a pure culture of a Gram-positive staphylococcus ; the remainder of the cultures were sterile.

Post-mortem Examination Results.

Three out of the 33 ducks examined had diseased ovaries very similar in appearance to a *B. pullorum* ovary in the fowl. One duck

was affected with chronic peritonitis. The remainder appeared perfectly healthy. Cultures made from the liver, spleen, and heart blood proved sterile in all 33 cases, except that the heart blood of one gave a few staphylococcus colonies. Ovarian cultures proved sterile in 28 apparently healthy ducks and in one duck with a diseased ovary. A second duck with a diseased ovary and the duck with peritonitis gave cultures of apparently contaminating organisms, as repeated plating revealed nothing but lactose fermentors. From one duck with a diseased ovary, and from one duck with an apparently healthy ovary, pure cultures of an organism apparently identical with Organism " B " were obtained. Further particulars are shown in Table IV.

General Remarks.

From the results of the serological tests, it would seem that the organism described by Gaiger and Davies (12) is a *B. enteritidis* type, and not *B. anatum*. In this respect, it is interesting to note that *B. anatum* has always previously been obtained as a kind of symbiant to *B. ærtrycke*, both in ducklings and in chicks.

The scale of the further investigations on Farm " B " scarcely permits definite conclusions to be drawn. In support of the theory of egg transmission we have the history of the epizootic, the fact that only very young ducks appeared susceptible, and the isolation of the organism from the ovaries of adult birds. Can we assume that an organism found in the ovary may also be found in the egg ?

The fact that perfectly healthy ducklings were hatched from the eggs of infected stock and the failure to obtain the organism from 100 eggs examined tend to contradict the egg transmission theory. It must be remembered, however, that neither of the ducks shown to be definitely affected may have layed during the investigations, or, as in pullorum disease, only a percentage of such eggs may contain the bacilli.

In brief, the investigations have only produced additional indefinite evidence for both sides of the question, which is one of considerable importance. Many *B. pullorum* infected eggs must be eaten daily, apparently with no ill-effects, but a *B. enteritidis* or *B. ærtrycke* infection would result in a different story. The subject is obviously one which calls for a very thorough investigation.

Conclusions.

1. The cause of three epizootics occurring in ducklings has been investigated. In one case an organism apparently identical with *B. enteritidis*, in the other two organisms closely allied to *B. ærtrycke*, were isolated.

2. A very thorough investigation of the possibilities of egg transmission of these organisms is being prosecuted as the question is one of importance. The results of investigations described in this article point to a distinct possibility of egg transmission.

3. In the author's opinion, *B. anatum* or keel disease has not yet been identified in England.

TABLE I.

Antigens.	*Sera.*													
	B. enteriditis.					*B. anatum (Gaiger).*					*Organism " B "*				
	Unabsorbed.	*Absorbed by*				*Unabsorbed.*	*Absorbed by*				*Unabsorbed.*	*Absorbed by*			
		B. enteritidis.	*B. anatum (Gaiger)*	*Organism " B."*	*B. pullorum.*		*B. anatum (Gaiger)*	*B. enteritidis.*	*Organism " B."*	*B. pullorum.*		*Organism " B."*	*B. enteritidis.*	*B. anatum (Gaiger)*	*B. pullorum.*
B. enteriditis	C 1 : 800	–1:100	tr1:100	–1:100	C1:600	C1:600	–1:100	–1:100	–1:100	C1:500	C1:500	–1:500	–1:100	–1:100	C1:400
B. anatum (Gaiger)	C1:800	–1:100	–1:100	tr1:100	C1:600	C1:600	–1:100	–1:100	–1:100	C1:600	C1:500	tr1:100	–1:100	–1:100	C1:400
Organism "B"	C1:800	–1:100	–1:100	–1:100	C1:600	C1:600	–1:100	–1:100	–1:100	C1:500	C1:500	–1:100	–1:100	–1:100	C1:500
B. pullorum	C1:700	–1:100	–1:100	–1:100	–1:100	C1:600	–1:100	–1:100	–1:100	–1:100	C1:500	–1:100	–1:100	–1:100	–1:100

C=complete macroscopic agglutination. tr=trace of agglutination. –=no agglutination.

Group I. absorption tests.

TABLE II.

Antigens.	*Sera.*									
	B. ærtrycke.					*Organism "F."*				
	Unabsorbed.	*Absorbed by*				*Unabsorbed.*	*Absorbed by*			
		B. ærtrycke.	*Organism "F."*	*Organism "S."*	*B. anatum 850.*		*Organism "F."*	*B. ærtrycke.*	*Organism "S."*	*B. anatum 850.*
B. ærtrycke	C1:1000	–1:100	–1:100	–1:100	d1:100	C1:1000	–1:100	–1:100	–1:100	–1:100
Organism "F"	C1:1000	–1:100	–1:100	–1:1000	d1:100	C1:1000	–1:100	–1:100	–1:100	tr1:100
Organism "S"	C1:1000	–1:100	–1:100	–1:100	d1:100	C1:1000	–1:100	–1:100	–1:100	–1:100
B. anatum 850	C1:800	d1:100	d1:100	–1:100	–1:100	C1:700	–1:100	–1:100	–1:100	–1:100

C=complete macroscopic agglutination. tr=trace of agglutination. d=distinct agglutination. –= no agglutination.

Group II absorption tests.

TABLE III.

Effect of Experimental Inoculations of Organisms " B " and " F."

Animals.	*Route.*	*Dose.*	*Organism " B."*	*Route.*	*Dose.*	*Organism " F."*
Rabbits	i/v	0·1 c.c.	Killed in 2 days. Pure cultures from tissues.	i/v	0·1 c.c.	Killed in 2 days. Pure cultures from tissues.
	s/c	0·5 c.c.	No effect.	s/c	0·5 c.c.	No effect.
Guinea-pigs	i/p	0·2 c.c.	Dead 2 days. Pure cultures.	i/p	0·1 c.c.	Dead 24 hours. Pure cultures.
	s/c	0·5 c.c.	Dead 8 days. Pure cultures.	s/c	0·5 c.c.	Dead 7 days. Pure cultures.
Fowls.		0·5 c.c.	No effect any route.	i/v	0·5 c.c.	Went sick but recovered.
Day-old chicks.	s/c	0·1 c.c.	Majority died with symptoms like B.W.D. Pure cultures.	s/c	0·1 c.c.	Essentially the same as Organism " B."
	per os.	0·5 c.c.	Did not appear to infect.	per os.	0·5 c.c.	About 25% infected.
4-6 week old chicks			No effect, any route.			About 50% died, 1/v or s/c routes. Organism recoverable pure.
8-day old ducklings	s/c	0·2 c.c.	No effect.	s/c	0·1 c.c.	All dead. Pure cultures.
	per os.		No effect.			
1-day old ducklings	s/c	0·2 c.c.	One batch all died.* Another batch remained healthy.			
3-week old ducklings	s/c	0·3 c.c.	Three died, but all cultures proved sterile.	s/c	0·2 c.c.	All died, and pure cultures obtained.

All inocula used were 24 hour broth cultures. *The conditions at the time of inoculation were very cold and wet.

TABLE IV.

DUCK POST-MORTEMS.

Duck.	*Agglutination Test.*	*P.M.*	*Cultures.*
1276	(*a*) Slight 1·25	Healthy	Pure Organism " B " from ovary.
	(*b*) Marked 1·25		
1175	(*a*) Marked 1·25	Diseased ovary.	Pure Organism " B " from ovary.
	(*b*) Marked 1·25		
1176	(*a*) Marked 1·25	Diseased ovary.	Lactose fermentors only from ovary.
	(*b*) Not done.		
1355	(*a*) Marked 1·25 (*b*) Marked 1·25	Diseased ovary.	Sterile.
1352	(*a*) Marked 1·25	Chronic peritonitis.	Lactose fermentors only from ovary.
	(*b*) Not done		

Test (*a*) approximately four months prior to death.
Test (*b*) approximately two months prior to death.

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SIX CASES OF CANINE CANCER.

By W. R. Spurrell.

(Department of Experimental Pathology and Cancer Research, University of Leeds).

In the course of an investigation into the influence of radium upon malignant disease in animals there was encountered a series of tumours presenting a striking similarity in clinical characteristics while varying considerably in histological detail. In view of the common occurrence of such tumours in certain breeds of dog the following account of these cases may be of interest.

The series consisted of six dogs, of which four were Airedales, one an Alsatian, and one a wire-haired terrier with black and brown markings. A feature common to all was the presence of a large amount of black or brown pigment in the skin, together with multiple small innocent cutaneous tumours, coal-black in colour and frequently referred to as " melanotic." All the dogs were over eight years of age and the malignant tumours were of recent occurrence.

The multiple pigmented tumours varied in size from a pea to a small grape ; they were generally pedunculated but a few flat plaques were occasionally found. The surface of the tumour was smooth, shiny and jet-black in colour. To the touch they possessed a peculiar silky softness of surface and a rubber-like consistency, characteristics very similar to those found in the tumours of human " neuro fibromatosis." The distribution was mainly on the inner aspects of the limbs and on the abdominal wall.

These primary tumours are essentially innocent. The usual procedure adopted by veterinary surgeons for their removal is the use of a snare and the results are reported to be very successful. In certain cases, however, removal has to be effected by means of the knife and in this series all the animals had been so treated. Here the results were not successful, for the wounds failed to heal and the tumours soon recurred in an obviously malignant form. At first sight it might be thought that the trauma of excision was responsible for the malignant change, and this view was expressed by some of the operators concerned. It seems more probable that the factor which made the surgeon decide to use the knife rather than the snare was evidence of early malignant change already present in the tumours. The size of the tumour, its encroachment upon surrounding tissue, and the lack of well-defined pedunculation would render the use of the snare difficult and these changes could reasonably be associated with early malignancy.

The animals were first seen by the writer at intervals of from six weeks to four months after the excision of the primary tumour and they all presented a picture of definite malignancy. A shallow ulcer

with hard, raised, and everted edges was present in each and a mass of dense tissue could be felt underlying the ulcer; attachment to deeper structures was not a striking feature. The regional glands were usually palpable. The general health of the dogs was but little impaired and there was no clinical evidence of secondary deposits in other organs. The four Airedales and the terrier were treated with radium alone. In all cases the tumours diminished markedly in size, but with the exception of Dog I this improvement was only temporary and the tumours soon resumed growth with such vigour that the animals had to be destroyed. In the case of Dog I the tumour disappeared completely and the skin healed; this dog was destroyed for other reasons six months later, and at post-mortem there was still some evidence of growth in the deeper structures underlying the site of the healed ulcer. In the case of the Alsatian, which possessed the largest tumour of the series, wide surgical removal was combined with radiation and the animal has remained well for 18 months.

A typical primary tumour, removed from Dog VI in this series, is a polypoidal mass of loosely-arranged spindle-cells covered by a thin layer of epithelium. Towards the centre of the tumour large areas are seen undergoing myxomatous change. At the periphery the cells are more densely packed and lie with their long axes parallel to the surface. The tumour-cells are pigment-free, but the sub-epithelial layer contains numerous phagocytes loaded with pigment, which is responsible for the black colour of the tumour as seen by the naked eye. The structure is that of a myxofibroma and bears a strong resemblance to that of the human neuro-fibroma, a finding quite in keeping with its clinical characteristics. There is no evidence that the tumour is connected with pigment-formation, so that the title " melanoma " is not justified.

Despite the close similarity in the clinical histories of these cases, the histological examination of the malignant tumours revealed considerable differences. The details of individual cases are given below and it will be seen that the series includes two malignant neuro-fibromata, a fibro-sarcoma, a spindle-cell sarcoma, a malignant melanoma and a leiomyo-sarcoma. The first four may possibly represent morphological variations of similar tissue, *viz.*, the terminal elements of small nerve fibres; the melanoma must be associated with the melanogenic apparatus whereas the leiomyo-sarcoma probably arose from an arrector pili.

Case Reports.

Dog 1.—*History*. Airedale, m., æt nine; multiple pigmented tumours. One excised from the right fore-limb four months previously. When seen the dog had a typical malignant ulcer 1½-in. in diameter at the site of operation; no glands were palpable. A portion was removed for section.

Histology.—The tumour consists of a mass of oval and spindle cells depositing strands of collagen throughout. Mitotic figures are numerous. The deeper layers of the epithelium are loaded with greenish-brown pigment

and a little is found between the tumour-cells. The tumour has the appearance of a fibro-sarcoma.

Dog 2.—*History*. Airedale, m., æt nine ; multiple pigmented tumours of the skin, one excised from the near hind-limb a few months previously. When seen, the dog had a large malignant ulcer 3½-in. in diameter and 1¾-in. deep at the site of operation ; no glands were palpable. A portion was removed for section.

Histology.—The tumour consists of bundles of spindle-cells arranged in a manner similar to that of a uterine myoma. No collagen is present. A fair number of mitotic figures are seen and the tumour is definitely malignant. No pigment is present in the tumour cells. The tumour has the appearance of a leiomyo-sarcoma probably arising in an arrector pili.

Dog 3.—*History*. Alsatian, m., æt ten ; a few pigmented tumours of the abdominal wall ; one excised from the left side of the sheath two months previously. When seen, the animal had a large fungating tumour, 3½-in. in diameter and 3½-in. high, involving the skin in front of the groin. The inguinal glands were palpable. The tumour was greenish-black in colour, of a very dense consistency but freely movable on the underlying tissues. The tumour with the adjacent glands was excised *en bloc*.

Histology.—The tumour consists of masses of cells, oval, spindle and polymorphous, with very little inter-cellular material. Finely granular brown pigment is present in and around the tumour cells and heavily pigmented chromatophores are conspicuous under the epithelium. The tumour has the appearance of a malignant melanoma. The inguinal glands show simple inflammatory changes.

Dog 4.—*History*. Airedale, m., æt eight. Multiple pigmented tumours of the skin, one excised from the side of the sheath two months previously. When seen the animal had a malignant ulcer, 2-in. in diameter, at the site of operation ; the inguinal glands were palpable.

Histology.—The tumour consists of spindle-cells arranged in compact bundles ; occasional whorls are seen, particularly towards the periphery. The tumour is malignant and has the appearance of a malignant neuro-fibroma.

Dog 5.—*History*. Black and brown terrier, m., æt eleven. Pigmented cutaneous tumour excised twice during the previous three months. When seen the dog had a recurrent nodule about the size of a filbert surrounded by an indurated area 2-in. in diameter.

Histology.—The tumour is a spindle-cell sarcoma with numerous areas of necrosis. It is well defined and lying within its boundaries are relics of lymphoid tissue which suggest that the tumour is a secondary deposit in a lymph-gland. A few phagocytes containing pigment are present in the tumour and in an adjacent healthy lymph-gland, but the tumour cells are pigment-free.

Dog 6.—*History*. Airedale, f. Multiple pigmented tumours of the skin, one excised from the inner side of the left thigh some months previously. When seen, the animal had a fungating ulcer 3-in. in diameter at the site of operation. The inguinal glands were palpable. After unsatisfactory radium treatment, the animal was destroyed and at post-mortem a secondary nodule was found in the lung.

Histology.—The tumour consists mainly of spindle-cells arranged in bundles running in all directions ; collagen is present in places. Mitotic figures are numerous and a few foci of necrosis are visible. Pigmented chromatophores are conspicuous in the sub-epithelial layers but the tumour-cells are devoid of pigment.

The nodule in the lung consists of spindle-cells arranged in very definite whorls ; in some instances these whorls appear to be continuous with small nerves. There is no pigment present.

The primary tumour appears to be a fibro-sarcoma, and in view of the secondary deposit it may be considered a neuro-fibroma undergoing sarcomatous change.

Summary.

Six cases of malignant disease in dogs are described. In each, the lesion was a recurrence following the excision of an apparently simple skin tumour, one of several present on the abdomen or inner aspect of the limbs. One such primary tumour, examined histologically, closely resembled the neuro-fibroma of man. The malignant tumours presented a diverse histological picture. In two cases the appearances were those of sarcomatous change in a neuro-fibroma. The remaining tumours were a malignant melanoma, a leiomyosarcoma, a fibro-sarcoma, and an undifferentiated spindle-cell sarcoma respectively. All the dogs were over eight years of age.

I am greatly indebted to Professor M. J. Stewart for his advice and assistance in the examination of the histological material.

REVIEWS.

Kompendium der Speziellen Pathologie und Therapie für Tierärzte. Published by Ferdinand Enke, Stuttgart, 1932. Price 23 Marks.

In the new edition of this well-known book the organ diseases are dealt with by Prof. Fröhner as in previous editions, while Prof. Zwick is responsible for the contagious diseases.

It is mainly designed for students, and the authors have exercised sound judgment in deciding what it is reasonable to include in such a work. In general, the amount of space devoted to each subject corresponds with the importance of the disease. The text is concise, clear, and up-to-date.

Filterable Virus Diseases in Man. By Joseph Fine, M.D., B.SC. Edinburgh : E. and S. Livingstone, 1932. Price 6s. net.

This small book (134 pages) has been designed in order to make accessible to senior medical students and interested medical practitioners up-to-date information regarding the extensive and important group of human diseases caused by what are usually called the ultra-visible viruses. It can be strongly recommended for that purpose.

For the purpose of description the author has grouped the filterable virus diseases according to their method of transmission. Divided in this way, they form six groups : (1) The yellow fever group ; (2) the typhus group ; (3) the dermotrophic group (affecting skin or mucous membrane) ; (4) the neuro-dermotropic group (affecting the nervous system and skin) ; (5) the neurotropic group (affecting the nervous system only) ; (6) the respiratory group.

In separate chapters the different viruses are described with regard to recent history, effect of physical and chemical conditions, filterability, pathogenicity for animals, vector, and immunology.

The author has made an exception to the rule that he would only include the essentially human diseases, for rabies is dealt with.

At the conclusion of each chapter there is a useful bibliography.

Die Streptokokkeninfektionen des Euters. By Dr. M. Seelemann. Published by M. and H. Schaper, Hannover, 1932. Price 14 Marks. Pages 259.

Dr. Seelemann is one of the best known of the earnest band of workers who have set themselves to study the ætiology and biology of the inflammatory diseases of the cow's udder, and he has done well to give, in this monograph, the fruit of his investigations up to the present time. The work can be most strongly recommended to both medical men and veterinary surgeons, as it is packed with information of the greatest interest.

It has gradually been recognised that, although there are different kinds of mastitis in the cow—that is, inflammations caused by different micro-organisms—the prevalent type is the one of which the description can be traced back in literature in Switzerland and Germany under the name of " gelbe Galt," and for which streptococci are responsible.

The information is arranged in separate chapters which deal with the history of the disease, its scientific importance, its causes, the methods of infection, the technique of milk examination, the organisms concerned in the

causation of the disease, the clinical characters, and, finally, its treatment and prevention.

A puzzling fact in connection with the great extension to which the disease has attained is, that it does not appear to be readily transmissible experimentally under what may be called natural circumstances, and the exact method by which the disease is spread in a cow shed has yet to be determined. In the author's experiments the application of infected milk or culture to the floor, teats, or udder, and even injection of infected materials in small quantity into the teat, failed to infect. The result in eighteen experiments in which different methods of infection were tried was positive only in one case, in which the bedding was kept infected with milk containing streptococci for about a month. In the author's opinion, even bad milking does not favour the infection, and apparently some unrecognised factor plays a critical rôle.

In a number of chemo-therapeutic experiments the author found that rivanol was of value in the treatment of mastitis. In many cases it appeared to abolish the streptococci from the udder. The treatment is carried out by injecting the affected quarter tensely full with an infusion, on two or three occasions, of a solution of 0·25 per mille. After the treatment, milking thrice a day helps the cure. The treatment was not successful in all cases, and unfortunately, it caused a serious fall in the yield of milk, which might last for some weeks. The best period for trying the treatment would appear to be while the cow is dry.

Inoculation with live or dead cultures of streptococci isolated from diseased udders have proved valueless either for prevention or cure and the author warns stockowners against its employment.

ABSTRACT.

THE IMPORTANCE OF SOLAR RADIATION IN THE DEVELOPMENT OF GROWING PIGS.

By A. M. Shaw.

To determine if possible the effect of Solar Radiation or direct light on young, growing pigs, an experiment was planned with light as the only variable. It was repeated four times, with the following results.

First Trial.

Four groups of 9 pigs each were selected for the trial. Each group was made up as follows : two pigs, 4 months of age ; and seven pigs, 2 months of age. Pigs from 5 litters were used and distributed as evenly as possible in the 4 groups. The pigs were of Yorkshire breeding. The experiment commenced on December 23rd, 1927, and continued for a period of 126 days. Direct light in the experiment refers to sunlight or skylight received by the animals without first being modified by transmission through glass or other absorbing medium.

Housing.—The sleeping quarters consisted of wooden buildings 9 ft. by 10 ft. and about 18 ft. high, equipped with plank floors and straw lofts. A yard 10 ft. wide and 18 ft. long with walls 4 ft. high was erected on the south side of each pen. Three of these yards were completely covered so that no light could penetrate into them. The fourth yard was not covered but simply enclosed by the 4 ft. fence. Feeding troughs were placed at the north end of each yard. To enable the attendant to feed the pigs in the covered pens, a drop door just above the trough was arranged so that it could be pushed inward sufficiently to allow the feed to be placed in the trough and yet not allow any light to enter.

Each pen had an opening 2 ft. by 3 ft. through which the pigs in each group could enter their respective yards. These outlets remained open during the entire trial so that all pigs had the same opportunity for exercise. All pens were kept clean and well bedded with straw, the attendant entering and cleaning pens through doors in the north wall.

The lighting of the pens was arranged as follows :—

Pen No. 1. (With open yard) had no glass in it.

Pen No. 2. (With covered yard) had 8 sq. ft. of ordinary window glass placed at such a height in the south wall as to flood the whole interior of the pen with light.

Pen No. 3. (With covered yard) had 8 sq. ft. of Vita glass placed in south wall in similar position as window in Pen 1.

Pen No. 4. (With covered yard) had no glass in it and was totally dark at all times.

The Vita glass used in this experiment was purchased from an authorised agent of the manufacturers of this product. It was also subjected to a laboratory test involving the use of the Spectrograph, by which it was found to transmit the ultra-violet rays down to 2,600 Angstrom units. This test was made in order to make sure that the glass used was a standard product.

Feeds.—All groups were fed exactly alike, the following feeds being used : oats, recleaned wheat screenings and wheat middlings. All grain was ground and hand fed dry twice daily with buttermilk as a protein supplement. Each group had access to a mineral mixture containing salt, lime, sulphur and charcoal.

Table I shows average gains made by all groups during period of 126 days. The two older pigs in each group have been listed separately from the seven younger pigs.

TABLE I.

Showing Average Gains made by all Groups during 126 Days. First Experiment.

Pen.	*Total number of Pigs.*	*Average initial weight lbs.*	*Average final weight lbs.*	*Average gain for 126 days.*
1. Open yard ...	2	82·0	251·0	169·0
2. Window glass...	2	84·5	253·0	168·5
3. Vita glass ...	2	93·0	254·5	161·5
4. Dark	2	79·0	237·5	158·4
1. Open yard ...	7	41·5	144·3	102·8
2. Window glass...	7	42·0	113·1	71·1
3. Vita glass ...	7	42·1	123·3	81·2
4. Dark	7	36·1	107·5	71·4

It will be noted from the above table that in the case of the pigs that were four months of age at the beginning of the experiment comparatively little difference in rate of gains occurred. All of them appeared to be able to develop about equally well under their respective conditions. The gains made by these pigs, however, can be stressed unduly because of the limited number of animals in each group, although the results are in general agreement with those found in the second experiment. These pigs up until the time they were selected for this experiment were kept under open yard conditions and fed a well-balanced ration. It would appear that a sufficient storage of Vitamin " D " had taken place before these pigs were placed on test to prevent the development of rickets during the period of the experiment. In the case of the younger pigs, however, a study of the gains made shows quite clearly that the seven pigs in Pen 1 (open yard) made decidedly better gains than those in Pens 2, 3 and 4.

Some observations made on the physical conditions of the pigs in the various groups are illustrated in the following table :—

TABLE II.

Showing Actual Physical Condition of all Pigs in each Group at the Conclusion of the First Experiment.

Pen.	*Total number of Pigs.*	*Number of Normal Pigs.*	*Slightly Affected with Rickets.*	*Number badly Affected with Rickets.*	*Number complete Cripples.*	*Percentage of normal Pigs.*
1. Open yard ...	9	9	0	0	0	100%
2. Window glass	9	4	1	3	1	44·4%
3. Vita glass ...	9	4	2	2	1 died	44·4%
4. Dark ...	9	3	2	3	1	33·33%

It is here clearly shown that some of the pigs in Pen 2, 3 and 4 were unable to develop normally, while, in Pen 1 all the pigs appeared to be normal in every way. The two oldest pigs in each group were in every case among those considered normal at the conclusion of the experiment. Only two pigs are listed as complete cripples, but it was quite evident that, had the experiments been carried much longer, most of those affected would have been listed as such.

Second Trial.

So definite were the results of this trial that it was decided to repeat it the following winter. The same equipment was used. The pigs selected for the second trial were Yorkshires. Litter mates were used as far as possible and distributed evenly throughout the first three groups. The pigs selected for the fourth group and kept in the pen with the open yard were only two months of age at the commencement of the experiment, December 16th, 1928. The trial had lasted for 120 days when it was decided to discontinue it on account of the very bad condition of a number of the pigs.

The following table gives a summary of the gains made by each group. No variation existed within the groups as in the previous experiment except that in the group which was kept in Pen 4, the dark pen, one pig somewhat older and larger than any of the others was included.

TABLE III.

Showing Average Gains made by all Groups during period of 120 Days. Second Experiment.

Pen.	*Total number of Pigs.*	*Average initial weight lbs.*	*Average final weight lbs.*	*Average gain for 120 days lbs.*
1. Open yard ...	8	38·2	138·5	100·3
2. Window glass...	8	44·5	125·7	81·2
3. Vita glass ...	8	44·25	118·2	73·95
4. Dark	8	44·0	125·0	81·00

It is again evident as in the former trial that the pigs in Pens 2, 3 and 4 were unable to make anything like normal gains, and that the pigs in Pen 1 with open yard easily outstripped them.

The pigs were carefully examined at the conclusion of the experiment with the following result :—

TABLE IV.

Showing Actual Physical Condition of all Pigs in each Group at the Conclusion of Second Experiment.

Pen.	*Total number of Pigs.*	*Number of Normal Pigs.*	*Slightly Affected with Rickets.*	*Number badly Affected with Rickets.*	*Number complete Cripples.*	*Percentage of normal Pigs.*
1. Open yard ...	8	8	0	0	0	100%
2. Window glass	8	0	3	2	3-1 died before close of experiment	0
3. Vita glass ...	8	0	3	2	3	0
4. Dark ...	8	1	3	2	2-both died before close of experiment	12·5%

It will be noted that none of the pigs in Pens 2 or 3 were normal at the conclusion of the experiment. In Pen 4, however, one normal pig was found. It was the pig referred to previously as being somewhat older than the others. This result is directly in line with the previous trial where, in every case, the older pigs were able to develop normally.

The first signs of crippling occurred in Pen 2 on March 8th. By March 25th, all showed unmistakable signs of rickets. On April 5th one died.

In Pen 3 crippling was first noticed on March 10th and by March 25th all the pigs in the group were affected.

In Pen 4, the first sign of crippling appeared on January 31st. One pig died February 26th and a second one on March 2nd. All the pigs in this group showed evidence of rickets, with the exception of the one older pig which remained apparently normal.

In Pen 1, all the pigs remained normal throughout the entire duration of the experiment.

Not only were the pigs in Pen 1 normal in each experiment, but it might be added that adjacent to the experimental pens both during the winter of 1927-28 and 1928-29 some 150 pigs of the same age and weight were kept in pens similar to No. 1 with entirely satisfactory results. Not a single case of rickets was observed among this rather large number of feeder pigs, indicating very clearly that light is of vital importance in the development of the growing pig.

Related Tests.—To secure further evidence that the absence of direct light was the cause of the rickets developing in the various groups, an additional test was made at the close of the first experiment. All the pigs

affected were placed in a colony house in a dry lot, fed exactly the same way but were at liberty to go in or out at will. Immediately it was noticed that all the pigs remained outside of the pen most of the time. They gradually improved until finally even the ones that at the beginning could not stand were able to walk stiffly to the trough. Eventually all recovered sufficiently to be marketed although many bent legs and enlarged joints were still in evidence.

At the end of the second trial, the covering of the yards attached to Pens 2, 3 and 4 was removed. Immediately the pigs took advantage of the light and remained outside the pen, stretched out on their sides most of the time. The skin soon commenced to take on a ruddy hue and, in a comparatively few days, an improvement in their physical condition was noticeable.

At first, some of these pigs were unable to use their hind quarters at all, and dragged themselves about with great difficulty. Others walked on their knees while others had enlarged joints or bent legs. All of them, however, slowly recovered the use of their limbs and gradually began to thrive. Those least affected soon began to gain rapidly in weight and even some of those pigs that were listed as complete cripples at the conclusion of the experiment eventually recovered sufficiently to be marketed.

Third Trial.

During the summer season of 1929, three lots of eight pigs each were placed in " A " shaped colony houses 8 ft. by 8 ft. square with covered yards 8 ft. by 18 ft. attached. In this test, the lighting of the houses was arranged as follows :—

Pen 1. An opening 4 ft. by 4 ft. in the south wall of house covered with wire netting (no glass).

Pen 2. Windows 4 ft. by 4 ft. of common glass.

Pen 3. Windows 4 ft. by 4 ft. of Vita glass.

The experiment commenced on June 28th, 1929, and continued for 145 days. The method of feeding was similar to that used in the previous trials with the important exception that all lots were fed on an abundance of fresh green alfalfa daily. The following table indicates the results obtained :—

TABLE V.

Showing Average Gain made by all Groups during period of 145 Days. Third Experiment.

Pen.	*Total number of Pigs.*	*Average initial weight lbs.*	*Average final weight lbs.*	*Average gain for 145 days lbs.*
1. Open window	8	28·37	185·	156·6
2. Window glass	8	25·25	166·8	141·6
3. Vita glass ...	8	24·9	163·4	138·5

No pigs in this test developed rickets, but the group in Pen 1 provided with the open window made decidedly better gains than the group in Pen 2 or Pen 3. Moreover, the difference between Pen 2 (common glass) and Pen 3 (Vita glass) was negligible ; in fact, was slightly in favour of the

group housed behind common glass. The failure of the pigs in this experiment to develop rickets would seem to have been due to the presence of Vitamin " D " in the fresh green alfalfa.

Fourth Trial.

As this result was slightly at variance with our previous findings, it was decided to run still another test. This trial commenced on December 12th, 1929. Two groups of eight pigs of uniform breeding, age and weight were placed in each lot. A part of the equipment used in the previous experiment was utilised. Pen No. 1 was provided with light through an opening in the south wall 4 ft. by 4 ft. covered with wire netting and Pen No. 2 received its light through a window of common glass of exactly the same size : *viz.*, 4 ft. by 4 ft. No Vita glass was used in this trial. The pigs were on test 130 days and, during the entire period, both groups were fed exactly alike. The results with regard to average gain appear in Table VI.

TABLE VI.

Showing Average Gains made by each Group during period of 130 Days. Fourth Experiment.

Pen.	*Total number of Pigs.*	*Average initial weight lbs.*	*Average final weight lbs.*	*Average gain for 130 days lbs.*
1. Open yard ...	8	39·16	148·5	109·4
2. Window glass...	8	38·37	123·6	85·3

The difference in rate of gain is again very distinctly in favour of the group in Pen 1. This difference in weight, however, does not tell the whole story as will be seen by an examination of Table VII.

TABLE VII.

Showing Actual Physical Condition of all Pigs in each Group at the Conclusion of Fourth Experiment.

Pen.	*Total number of Pigs.*	*Number of Normal Pigs.*	*Slightly Affected with Rickets.*	*Number badly Affected with Rickets.*	*Number complete Cripples.*	*Percentage of normal Pigs.*
1. Open yard ...	8	8	0	0	0	100%
2. Window glass	8	0	5	2	1	0%

All pigs in Pen 2 were affected, one of them being a complete cripple, and all of the others showed unmistakable signs of rickets.

The pigs in Pen 2, by the end of the experiment, were quite definitely on the down grade, while the pigs in Pen 1 were perfectly healthy and had every indication of being in a thriving condition. Owing to the fact that this experiment was carried on during the winter months, the group in Pen 1 was exposed to a much greater extent than those in Pen 2. The temperature in Pen 1 was, at all times, practically the same as the temperature outside,

on many occasions the thermometer registering as low as 30° F. below zero. In spite of this apparent handicap, these pigs remained in much better physical condition than the pigs in Pen 2, which appeared to be much more comfortable in every way.

SUMMARY.

The results obtained would seem to show :—

1. That direct light is necessary for the normal development of pigs.
2. That young pigs fed what would ordinarily be called well balanced rations but deprived of light will develop rickets.
3. That similar young pigs fed in the same manner but having access to open yards or direct light will remain normal.
4. That the light secured through ordinary window glass is of little value in preventing rickets in young pigs.
5. That Vita glass is of very doubtful value. Certainly if superior at all, it is not sufficiently superior to ordinary glass to justify the great difference in price.
6. That, by furnishing an abundance of fresh green legumes or probably other green plant growth, the occurrence of rickets will be prevented.
7. That young pigs show the effect of restricted light much more quickly than older ones.
8. That pigs 3 or 4 months of age that have up to that period developed normally seem to be able to withstand comparatively long periods of restricted light.
9. That access to direct light will cause rapid improvement in affected animals and eventually restore pigs showing evidence of most advanced cases of rickets sufficiently to be marketable.
10. That entirely apart from the development of rickets, the pigs which received direct light made significantly greater gains than all other groups in the experiment.
11. That, in view of the results obtained in this experiment, it would appear that the most satisfactory system of housing for young growing pigs the year round is to provide them with comfortable sleeping quarters with open yards attached to which they can have access at all times. (*Scientific Agriculture*, Vol. XI, No. 1, 1930.)

CONTENTS.

GENERAL ARTICLES.

PAGE

Studies on Bovine Mastitis. VII.—The Serological Characters of Mastitis Streptococci. By A. W. Stableforth 185

Gas Gangrene infections of Sheep: Passive immunisation. By A. D. McEwen and R. S. Roberts 212

Comparison of Repeated Rapid Whole-Blood, Rapid Serum, and Tube Agglutination Tests for the Diagnosis of *S. Pullorum* Disease. By Jacob Biely and William Roach 224

The Effect of Spirochete and Trypanosome Infection on the Distribution of Chemotherapeutical Drugs. By Alexander Hasskó, V.D. 230

Studies in Louping-Ill (An Encephalomyelitis of Sheep). II.—Transmission by the Sheep Tick, *Ixodes ricinus* L. By J. MacLeod, B.SC., PH.D., and W. S. Gordon, M.R.C.V.S. 240

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